Understanding Population Variability in Age-at-Death Estimation for Modern Populations in Mexico and Puerto Rico Through the Use of 3D Laser Scans of the Pubic Symphysis

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ABSTRACT

Reliable age-at-death estimation from the adult skeleton is of fundamental importance in forensic anthropology, as it contributes to the biological profile estimations in a medico-legal death investigation. However, reliable estimates are challenging because many traditional aging methods are dependent upon a set of population-specific criteria derived from individuals of European and African descent. Potential differences in the aging patterns of diverse populations may hinder our efforts to produce useful age-at-death estimates in underrepresented groups. In response to these concerns, this study explores the utility of currently available aging techniques, and explores the need, if any, for population-specific aging methods among groups from Latin America.

The current study obtained data from two skeletal collections representing modern individuals of Mexican and Puerto Rican origin. Five newly developed computational-shape based techniques utilizing 3D laser scans of the pubic symphysis and one traditional bone-to-phase technique were examined. A validation test of all computational and traditional methods was implemented, and new population-specific equations using the computational algorithms were generated and tested against a Spanish sample. Estimated mean ages from the traditional and computational techniques were compared in order to offer practical recommendations for age estimation for cases of Latin American identity and, in particular, cases presumed to be of Mexican or Puerto Rican ancestry.

Results from this study suggest that traditional and computational aging techniques applied to the pubic symphysis perform the best with individuals in the 35-45 age group, in comparison to other age groups. Levels of bias and inaccuracy increase as chronological age increases, with overestimation of individuals under 35 years of age and underestimation of individuals over 40 years of age. New regression models provided comparable error rates to, and in several occasions, outperformed the original computational models developed on White American males. Ultimately, however, age estimates did not significantly improve. This study has shown that population specific models do not necessarily improve age estimates in samples from Latin America. Results do suggest that computational methods can ultimately outperform the Suchey Brooks method and provide improvement in objectivity when estimating age-at-death in samples originating from Latin America.

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CHAPTER ONE
INTRODUCTION

Age-at-death estimation techniques have received considerable attention within the anthropological community, especially among forensic anthropologists, as correctly interpreting age-at-death for unknown human remains can contribute probabilistic evidence towards a positive identification in the medico-legal setting (Algee-Hewitt 2017; Garvin and Passalacqua 2012; Konigsberg et al. 2008; Steadman et al. 2006;). Accordingly, age at death is an important component of the biological profile, which also requires the estimation of other identity parameters such as sex, stature, and ancestral background for the skeletonized or fragmentary remains under analysis. Age-at-death estimation is among the most critical features of this biological profile (Algee-Hewitt 2015), as it can decrease the number of possible matches for the medical examiner and reduce the investigative burden for law enforcement. However, the estimation of age based on skeletal remains is one of the most challenging biological parameters to estimate. This is in part due to the complex biological variability in aging patterns between and within populations (Kemkes-Grottenthaler 2002), as well as the methodological and observer-related biases associated with many of the traditional morphological bone-to-phase techniques currently available in the field (Kemkes-Grottenthaler 2002; Kimmerle et al. 2008; Lottering et al 2013; Shirley and Ramirez Montes 2015; Usher 2002).

When estimating age-at-death, anthropologists have relied on life senescent (or degenerative) changes in the skeleton, including wear and tear of joint surfaces, which can start occurring after the second or third decade of life (Brooks and Suchey 1990; Todd 1920; Buckberrry and Chamberlain 2002; Lovejoy et al. 1985). Forensic anthropologists utilize multiple morphological features of the skeleton to estimate age-at-death, including the pubic symphysis (Slice and Algee-Hewitt 2015; Brooks and Suchey 1990; Katz and Suchey 1989; Stoyanova et al. 2017;Todd 1920), the auricular surface of the ilium (Buckberry and Chamberlain 2002; Lovejoy et al. 1985; Osborne et al. 2004), the sternal end of the fourth rib (Iscan et al. 1984a,1984b; Iscan et al. 1985), and cranial sutures (Meindl and Lovejoy 1985; Nawrocki 1998).

According to a recent study by Garvin and Passalacqua (2012), the pubic symphysis is the preferred and most widely used skeletal element by forensic anthropologists when estimating age-at-death. Even though many age-at-death techniques focusing on the pubic symphysis have been created, they are not equal in terms of their applicability or utility in a medico-legal setting. For example, Valencia-Caballero and colleagues (2015) presented evidence that a sample of pubic symphyses of Mexican males was misclassified (i.e., overestimation) 50% of the time when using the Suchey Brooks method. In contrast, Hens et al. (2008) demonstrated that when the Suchey Brooks method was applied to an Italian sample, accuracy of age-at-death estimates were comparable to age estimates of samples of American and African American ancestry. In a similar study, Schmitt (2004) suggested that the Suchey Brooks method was a poor predictor of age for an Asian sample. The lack of agreement between studies examining the same technique demonstrates that estimating age from the pubic symphysis is a complicated task. This is due to several factors, including (1) the potential morphological variation in the senescent process between and within populations, and (2) methodological and observer-related biases associated with each aging technique.

Age-at-death estimation is possible because a correlation exists between chronological and biological age. Chronological age is a measure of time since birth (i.e., years, months, days), whereas biological age is a physiological state that is affected by multiple variables, such as genetic makeup, diet, climate, physical activity, socio-economic status, and overall health. Yet, this correlation is imperfect, as each individual will display differences in the magnitude and rate of senescent in their skeleton under different life conditions (Kemkes-Grottenthaler 2002). Furthermore, the strength between the correlation of chronological and biological age decreases as age progresses, due, at least in part, to the widening of the range of variation in the morphological changes observed in the age informative areas of the skeleton. For this reason, it is said that each skeleton has its own degree of error (Boldsen et al. 2002).

Age estimation can be especially difficult in forensic contexts when the unidentified skeleton is not sourced from a known population. Methodological challenges to estimate age-at-death arise from the fact that most of the established aging techniques were derived from individuals of European, and to a lesser degree, African ancestries from late 19^{th} and mid-20^{th} century anatomical collections. Since many of these techniques are based on relationships between trait expression and age that were defined using a particular reference sample, there is potential for error when examining individual members or samples of populations who are temporally, geographically, genetically, environmentally and morphologically different from the reference collections. Moreover, whether adopting the more conventional bone-to-phase matching approach (Brooks and Suchey 1990; Buckberry and Chamberlain 2002; Iscan et al. 1984a, 1984b; Iscan et al. 1985; Katz and Suchey 1989; Lovejoy et al. 1985; Osborne et al. 2004; Todd 1920) or implementing a more computational technique that relies upon statistical modeling techniques for data collection and estimation (Konigsberg 2015;Slice and Algee-Hewitt 2015; Stoyanova et al. 2015; Stoyanova et al. 2017), the quality of the final age result depends on the forensic anthropologist's ability to identify and have available the most appropriate reference sample against which to compare the unknown. This issue is especially concerning when attempting to estimate age-at-death for Hispanic individuals.

According to the 2010 U.S. Census and the United States Office of Management & Budget (OMB), Hispanics or Latinos are defined as individuals originating from Mexico, Puerto Rico, Cuba, South and Central America, or any other Spanish speaking country who reside in the U.S. (Ennis et al. 2010). The Hispanic category is widely used within the anthropological community, yet the criteria used to classify these individuals (i.e., language, geographic location) have no biological basis and such terminology ignores the complex and diverse ethno-histories of the many groups included in this category (Ross et al. 2004). As a multi-group category, Hispanic encompasses peoples who represent complex genetic or ancestral histories that have often been shaped by very different periods of migration and colonization (Via et al., 2010; Martinez-Cruzado et al., 2008; Rangel-Villalobos et al., 2008; Rubi-Castellanos et al., 2009). Present day Hispanic groups exhibit complex population structures with significant ancestral contributions from Europeans, Native Americans, and West Africans (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Gonzalez Buchard et al. 2005; Hayes-Bautista 1980; Hughes et al. 2018; Ross 2004). Furthermore, existing research suggests that the multiple populations in this category could be sufficiently different, genetically and morphologically, to the extent of being distinguishable between groups (Ross 2004; Gonzalez Buchard et al 2005). The historical events that contributed to the complex patterns of genetic variation within and between Hispanic groups resulted in large heterogeneous populations, with extensive variation not only across geographical regions, but also within countries. For example, in a study examining five groups referred to as Hispanic, Puerto Ricans and Dominicans showed the largest proportions of African ancestry, in contrast to Mexicans, who had a significant contribution of Native American ancestry (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Gonzalez Buchard et al 2005; Hughes et al. 2018). Even within small islands in the Caribbean, there is evidence of high variance admixture proportions. Simms et al. (2010) examined the allelic frequency data of individuals from Haiti and Jamaica, and results show evidence that although both Haiti and Jamaica display genetic affinities closely related to African descent, Haiti had a stronger signal for African ancestry than individuals from Jamaica. These results are an example as to how divergent genetic and morphological data present in Latin American populations can allude to the different migratory events of West Africans and Europeans into the Americas.

In relation to morphological variation between Hispanic groups, there has not been a systematic examination of morphological variation among a broad spatial distribution of Latin America. Most research has focused on localized studies and has used a single population group to explain patterns of variation of different geographical regions (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Dudzik and Jantz 2016; Hughes et al. 2018; Ross 2004). This is mostly due to the lack of modern skeletal data from different geographical regions in Latin America. A few studies examining variation between groups, include Ross (2002), who was able to examine various groups from Latin America, including modern Cubans, Peruvians, and Mexicans, and provided evidence that there is cranial variation between these groups. In addition, Algee-Hewitt (2018) was able to examine craniometric data from Hispanic cases sourced from the Forensic

Data Bank (FDB,) and demonstrated that the quantity of Native American ancestry has increased in recent years (Algee-Hewitt et al. 2018). Furthermore, when using craniometric data, Algee-Hewitt and colleagues (2018) demonstrated that the distribution of Native American ancestry follows a northwest-to-southeast geographic cline originating from Mexico.

Despite the increasing share of Hispanics in the U.S. demographic in particular, reference samples for Latin American groups within the field of forensic anthropology are scarce. Consequently, population-specific standards for this category are very limited. Only recently have studies sought to produce information on estimation criteria for individuals of Mexican nationality (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Dudzik and Jantz 2016; Hughes et al. 2018; Spradley et al. 2008; Tise et al. 2013). Yet, research has been constrained to the study of sex and ancestry using metric skeletal data from positively identified US-Mexico border crosser fatalities (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Dudzik and Jantz 2016; Hughes et al. 2018; Spradley et al. 2008; Tise et al. 2013). Due to the small sample size and lack of demographic information (i.e., know chronological age) for these highly targeted groups, other parameters requiring osteological estimation, such as age-at-death, have not been thoroughly examined and validated against the most well-established aging techniques. Furthermore, it is still unknown whether or not a population specific approach for age-at-death estimation is needed at all for the Hispanic category as a whole, relative to other major populations of forensic interest in the U.S.

Acknowledging these issues, it is critical to ask whether aging techniques produced using skeletons of European and African descent are truly appropriate for aging peoples of Mexican and Puerto Rican origins. If not, does the potential mismatch between target and reference samples produce ill-fitting models and introduce additional sources of error in estimation? Furthermore, to what degree do any such inter-population differences affect final age estimates? To address these questions, one of the main goals of this study is to investigate if significant differences in age-at-death estimation are evident between two of the largest groups considered Hispanic in the United States: Mexicans and Puerto Ricans. The importance of examining groups of Mexican and Puerto Rican origin stems from the fact that their patterns of senescence are poorly understood. It is known that Mexican and Puerto Rican groups have completely different population histories, which could lead to genetic differences that may affect the expression of aging in bone. Furthermore, the applicability of common age-at-death techniques has not been thoroughly investigated, and rates of error for current techniques have not been reported. It is crucial that efforts are made to reach a clear understanding of age-related changes on these populations, to quantify the magnitude of error and bias when estimating age-at-death, and in turn, determine if population specificity in age-at-death estimation is needed.

Currently, the estimation of age greatly depends on the nature of training and expertise of the anthropologist to determine age-related changes in the pubic symphysis, and to visually compare those age-related changes with comparative photographs or casts representing an age range, previously recorded from a group of individuals with known ages (Baccino and Smith 2006 ; Brooks and Suchey 1990; Katz and Suchey 1989; Kimmerly et al. 2008; Slice and Algee-Hewitt 2015). The well-known problems of subjectivity and increased observed error on traditional age-at-death methods was highlighted in a recent survey of forensic anthropologists, where close to half of the respondents in a survey indicated that the choice to use age-at-death estimation from the pubic symphysis using traditional methods, and preferentially the Suchey

Brooks technique, was mainly dependent on expertise and experience rather than superior methodology (Garvin and Passalacqua 2012). In order to overcome this issue of subjectivity, several new age-at-death techniques have been established (Boldsen at al. 2002; Dudzik and Langley 2015; Milner and Boldsen 2012), including the Slice-Algee-Hewitt Score method (SAH), the Thin plate spline/Bending Energy method (TPS/BE), and the Ventral Curvature (VC) method (Slice and Algee-Hewitt 2015; Stoyanova et al. 2015; Stoyanova et al. 2017). These techniques together are novel for the fact that they provide a framework that is fully computational by quantifying the surface complexity of the bone, as computed from 3D laser scans of the pubic symphysis, and modeling the relationship between morphological shape and chronological age, with the end goal of providing an estimate of age for each skeleton. By eliminating the traditional gross morphological assessment of visually comparing age-related changes with comparative photographs or casts, and by applying robust shape-based algorithms and building regression equations for age determination, this computational framework seeks to reduce the high degree of variation in method interpretation and low degree of standardization across application and practitioners (Slice and Algee-Hewitt 2015; Stoyanova et al. 2015; Stoyanova et al. 2017). Even though this novel methodology has proven to be a promising tool for the rigorous quantification of age-at-death in European-Americans, its value has not been evaluated from a forensic perspective for the diversity of contemporary Hispanic populations, which are typically underrepresented in the literature on aging techniques available to forensic practitioners in the United States.

Current study

Responding to the aforementioned concerns regarding the utility of currently available aging techniques, and the need, if any, for population-specific aging methods among groups from Latin America, the current study focuses on age estimation from the pubic symphysis for samples of Mexican and Puerto Rican origin, considering primarily the preferred and most widely used traditional age-at-death estimation technique, the Suchey-Brooks method, and the computational shape-based techniques utilizing 3D laser scans introduced by Slice and Algee-Hewitt in 2015 (the Slice-Algee-Hewitt Score method (SAH), the Thin Plate Splines/Bending Energy method (TPS/BE), and the Ventral Curvature (VC) method). The current study explores two main goals. The first goal seeks to provide information concerning how well age estimation techniques (traditional and computational) yield accurate age estimates for Mexican and Puerto Rican samples. The second goal explores the potential for population specificity in age-at-death methods within groups of Latin American origin, and if they are necessary to improve accuracy in age estimation in a medico-legal setting.

In order to achieve these goals, the proposed project obtained data from several skeletal collections located in Latin America, specifically Mexico and Puerto Rico. A validation test of each published methodology was implemented on each group, and population-specific equations for individuals of Hispanic origin were created using the computational protocols previously published by Slice and Algee-Hewitt (2015) and Stoyanova (2015;2017). To determine if group-specific or universal equations are more appropriate when examining Hispanic groups, even when broadly defined, a large sample from Spain (i.e., Spanish speaking country) was included in the group analysis. In addition to examining how well current age-at-death estimation techniques for the pubic symphysis yield accurate age estimates, and the effects of group-specific and individual life history differences in senescence; the current study tested for asymmetry and identified if both elements of the pubic symphysis yield a similar age-range and quantify both the degree of intra- and inter- observer error associated with each aging method. Results derived from both traditional and computational techniques were compared for all three samples in order to offer practical recommendations for age estimation in forensic anthropology for cases of Hispanic identity and, in particular, those presumed to be of Mexican or Puerto Rican origin.

The goals and aims of the study are further elaborated in the following two hypotheses;

Hypothesis 1

It is hypothesized that the Suchey-Brooks method, which was originally developed on a multi-ethnic sample (i.e., Europeans and to a lesser degree, African and Mexican American samples), and the three newly computational methods (SAH Score method, TPS/BE method, and the VC method) of the pubic symphysis will yield age estimates with higher error rates for Hispanic samples than when applied to individuals of European and African ancestry, as the methods were originally developed on non-Hispanic samples. It is worth noting that the computational methods were only developed on a sample of 94 White males from the United States. This examination will help quantify the magnitude of error between all techniques and provide information as to how appropriate it is to use each method on several Hispanic samples. If age estimates with higher error rates are encountered when using traditional and computational methods on Mexican and Puerto Rican samples, then hypothesis (1) is expected to be supported and new population specific age estimation techniques should be taken into consideration when attempting to estimate age on samples of Mexican and Puerto Rican origin.

Hypothesis 2

Based on findings from population genetic studies (Gonzalez Buchard et al. 2005; Martinez-Cruzado et al., 2008; Rangel-Villalobos et al., 2008; Rubi-Castellanos et al., 2009;Via et al., 2010) and recent studies on ancestral variation from craniometric data (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Ross et al. 2002; Ross et al. 2004;) suggesting intragroup variation between Hispanic labeled groups, it is hypothesized that age estimation models developed for Hispanic samples will perform better if they are population specific. This hypothesis will address whether Mexican and Puerto Rican samples should be treated as two different populations, and in turn, require two different age-at-death techniques, or if they should be pooled together in order to develop a single age-at-death technique. This hypothesis will further answer questions related to the effects of population history and individual life history differences in senescence and its consequences to the morphological expression of the pubic symphysis.

Chapter organization

Chapter Two provides a historical background of age-at-death estimation methods for traditional and computational methods on the pubic symphysis. The chapter includes a brief summary of validation studies, limitations to each technique, as well as considerations when attempting to estimate age on the skeleton from a statistical point of view. Chapter Three includes an extensive review of the population history of Mexico and the Caribbean and highlights their shared ancestral history and how this history shaped the Hispanic term currently used in the United States.

Chapter Four provides a thorough description of the skeletal samples and statistical analysis used to test the aforementioned hypothesis. This chapter includes an extensive description of both traditional and computational methods used in the current study. Chapter Five and Six present the results obtained for each aging technique and include newly developed equations for age estimation in forensic anthropology for cases of Hispanic identity. Chapter Seven provides a summary of the results and includes a discussion of each hypothesis and how the results prove or disprove each hypothesis. In addition, appropriate recommendations for modifications to these techniques and best practices applications to forensic cases are included in this chapter. In conclusion, Chapter Eight highlights the main findings of the study, limitations, and recommendations for the estimation of age on Hispanic samples in a medico-legal setting.

CHAPTER TWO
AGE ESTIMATION FROM THE HUMAN SKELETON

The reliable estimation of age-at-death from the human skeleton is of fundamental importance to biological anthropology, with special interest to the field of forensic anthropology. Accurate age-at-death estimates are essential for the creation of a biological profile, which in turn contributes to the positive identification of unknown skeletal remains in a medico-legal setting. Obtaining accurate and precise age estimates are imperative, but nevertheless are a challenge to attain, mainly due to biological and methodological sources of error (Boldsen et al. 2002; Rissech et al. 2006; Lottering et al. 2013; Shirley-Langley and Jantz et al. 2010). Several of these sources include the lack of appropriate adult osteological reference collections with uniform distribution of ages (Hoppa 2002; Usher 2002; Kemkes-Grottenthaler 2002) and the lack of age-at-death techniques that include a robust statistical approach (Boldsen et al. 2002; Slice and Algee-Hewitt 2015). In spite of new innovative research addressing these concerns (Boldsen et al. 2002; Rissech et al. 2006; Lottering et al. 2013; Slice and Algee-Hewitt 2015; Stoyanova et al. 2017), the estimation of age-at-death from the adult skeleton is still inherently flawed, mainly due to methodological biases, observer error, and senescent variability between individuals.

This chapter will provide a theoretical foundation to better understand the basis of the hypothesis that current traditional and computational aging techniques will yield higher error rates when used on individuals who are genetically and morphologically different from the reference sample used to create the technique. As this study is concerned with the estimation of age from the pubic symphysis, it is imperative to include a general review of the evolution of age-at-death estimation of the pubic symphysis, with an emphasis on traditional and computational techniques. The aforementioned issues influencing the accuracy and precision of age estimation will be discussed in more detailed, highlighting several validation studies and the recent advancements within the field.

Basis of age-at-death estimation

Traditional age-at-death estimation techniques on the adult skeleton are mostly based on changes associated with growth and development, specifically degenerative and morphological changes across skeletal features of the adult skeleton (Algee-Hewitt 2013; Suchey and Katz 1998; Katz and Suchey 1989; Lovejoy et al. 1985; Bullock et al. 2013). Even though it has been shown that the adult skeleton undergoes a “life-long age-related transformation” (Kemkes-Grottenthaler 2002, p.48), each element of the adult skeleton transforms in its own unique way (Buckberry and Chamberlain 2002). Furthermore, previous publications have demonstrated that the process of aging is dependent on a myriad of extrinsic and intrinsic factors, including distinctive genetic differences between and within populations, socioeconomic status, and lifestyle (i.e., access to food and healthcare, occupation) (Lottering et al. 2013; Meindl et al. 1985; Rissech et al. 2006; Schmitt 2004; Sinha and Gupta 1995; Shirley-Langley and Jantz et al. 2010). Thus, it is appropriate to state that each skeletal element or biological age marker does not represent chronological age, but merely an estimate of the physiological status of the individual (Buckberry and Chamberlain 2002).

In contrast to the estimation of age on the adult skeleton, which is dependent on the degenerative process (i.e., wear and tear) of the skeleton, the assessment of age from the juvenile skeleton is generally estimated by examining well-defined milestones that the skeleton and dentition undergo throughout the developmental period (Lewis and Flavel 2006). Many, if not all of these milestones are known to be under tight genetic control (Bogin 1999; Lewis and Flavel 2006; Stinson 2012). Therefore, these milestones have been used in the creation of standards to aid with the assessment of age, and many of these methods are known for being more accurate than current aging techniques on the adult skeleton.

While the rate at which the biological development of age related morphological changes is different, the general pattern of change should be the same. Several studies examining age-related morphological variability between individuals have demonstrated that the change in various age traits (e.g., pubic symphysis, auricular surface of the ilium) is different between males and females (Berg 2008; Lottering et al. 2013; Sinha and Gupta 1995; Katz and Suchey 1986; Mckern and Sterwart 1957) and has been shown to be different when applied to individuals or populations with a background (i.e., life history, diet, access to health care) different from that of the reference sample used to establish the technique (Bullock et al. 2013; Hoppa 2002). As such, differences in age-related changes in the skeleton may impede the use of these skeletal features on skeletal samples that differ significantly in time from the reference sample (Hoppa 2002).

Early research into skeletal biology focused exclusively on single trait morphology for the estimation of age (e.g., pubic symphysis and sacroiliac joint), but with continuous validation and modification of existing aging techniques, as well as the development of new methods utilizing multiple traits and the incorporation of computational techniques, the field of forensic anthropology has seen a dramatic shift in how age is estimated.

A wide array of skeletal features have been promoted for the estimation of age on the adult skeleton, but the most commonly known and used age indicators within the field include the pubic symphysis (Algee-Hewitt 2015; Brooks and Suchey 1990; Stoyanova et al. 2017;Todd 1920), the auricular surface of the ilium (Buckberry and Chamberlain 2002; Lovejoy et al. 1985; Osborne et al. 2004), the sternal end of the fourth rib (Harnett 2010; Iscan et al. 1984a,1984b; Iscan et al. 1985), and cranial sutures (Meindl and Lovejoy 1985; Nawrocki 1998). Previous studies have demonstrated that the pubic symphysis (anterior midline joint between the left and right os coxae) is the most frequently used (Aykroyd et al. 1999; Garvin and Passalaqua 2012) and most reliable technique for age-at-death estimation (Slice and Algee-Hewitt 2015). This is mainly due to the simplicity of current methodologies, which are based on the application of phase-to-age techniques utilizing the visual comparison of the symphyseal face morphology to photographs or casts with text-based descriptions (Suchey-Brooks 1990; Katz and Suchey 1989; Todd 1920; Brooks 1955; McKern and Stewart 1957). Furthermore, due to its location within the pubic bones, the pubic symphysis is protected by hyaline cartilage from many destructive taphonomic processes, making it more frequently available than other skeletal elements at the time of recovery. As this study focuses on the utility of the pubic symphysis on Hispanic populations, it is crucial to review the biology of the pubic symphysis, and how these biological processes allow for the estimation of age on the adult skeleton.

The pubic symphysis forms the anterior joint between the left and right os coxae. The articular surfaces of the symphysis are oval in shape and covered in hyaline cartilage. The pubic symphysis is subjected to a variety of forces during daily activities, including traction on the inferior aspect of the symphysis, compression against the hyaline cartilage on the superior aspect when standing, and shearing that forces both pubic symphysis in different directions during a single leg stand (Becker 2010; Buckberry 2015). Even though several forces act on the pubic symphysis, in comparison with other pelvic joints (sacroiliac joints, lumbosacral joint, hip joint), the pubic symphysis is only capable of a small amount of movement under normal physiological conditions (Westcott and Drew 2015).

In late adolescence, the subchondral bone of the symphyseal face has an antero-posteriorly oriented ridge and furrow system (Becker 2010; Suchey-Brooks 1990; Todd 1920; Brooks 1955). During early adult life, bone is deposited on the symphyseal face, thickening the cortical bone, and eventually obliterating the ridge and furrow morphology (Suchey-Brooks 1990; Todd 1920; Brooks 1955). This stage is also characteristically known for a slightly raised rim of bone deposition (Suchey-Brooks 1990; Todd 1920; Brooks 1955), expanding from the anterior to the distal aspect of the face. In adulthood and later-adulthood, the surface of the symphyseal face undergoes irregular erosion and the cortical bone starts to diminish, producing micro- and macro-porosity and an irregular surface (Suchey-Brooks 1990; Todd 1920; Brooks 1955). The aforementioned changes of the pubic symphysis are often referred to as degenerative and sequential, and these progressive changes are used in the formulation of literature standards to aid with the assessment of age.

Traditional skeletal aging techniques: Pubic symphysis

The first systematic method using the pubic symphysis as an indicator of age-at-death was created by T. Wingate Todd (1920) in which he studied age related changes in the symphyseal face of the pubic symphysis for both males and females. Todd (1920) utilized a skeletal collection housed at Western Reserve University in Cleveland, Ohio. The sample was originally composed of 306 White males ranging from 18 to 88 years of age. Sometime later, a second sample was added to the original reference sample (90 Black males, 47 White females, and 20

Black females). Todd was able to demonstrate that as age increases, progressive degenerative changes are more evident in and around the symphyseal face. The morphological patterns described in his work include the antero-posteriorly furrows and ridges, the ventral beveling, the formation and sometimes completion of the dorsal and ventral margin, the demarcation of the upper and lower aspects of the symphyseal face, followed by a rim formation, and overall breakdown of the symphyseal face.

Todd utilized these observations to publish a 10-phase method for the estimation of age-at-death based on scoring criteria from the aforementioned morphological patterns of the pubic symphysis (Todd 1920; Todd 1921). The idea behind the technique was for the practitioner to identify which of the 10 phases truly represents the skeletal characteristic observed in the unknown sample. The assumption of Todd's method was that these progressive changes happening in the symphyseal face were progressive and uniform on every single individual, regardless of ancestral background. However, several issues with his method were highlighted in subsequent publications (Suchey-Brooks 1990; Brooks 1955; Katz and Suchey 1989; McKern and Stewart 1957). Some of these issues highlighted included 1) a narrow age range, 2) descriptions that are too specific and difficult to apply to other pubic symphysis that do not conform to the descriptions, 3) limited age-ranged samples, 4) lack of testing variability between ancestral groups, 5) biased estimates by excluding individuals from the reference sample that did not conform to the original descriptions, and 6) inaccurate age reporting for the reference sample.

Subsequent work by Brooks in 1955 found that Todd's method overestimated age for both males and females and proposed modifications to the general framework (Brooks 1995). Brook was able to re-examine a small sub-sample originally used by Todd, and a small archeological sample composed of males and females with known ages. Brooks (1955) demonstrated that Todd's method consistently overestimated age on both males and females, and young and older adults. Brooks proposed that the age phases be modified according to the limits proposed by statistician M.S Ahmed, so as to avoid overlapping of the phrases and to solve the issue of under-aging. For example, it was recommended that the age limits for phases V through VIII be modified some 3 years younger in order to solve for issues of underestimation. Following Brooks' analysis, McKern and Stewart (1957) proposed further refinement to the ten-phase method originally published by Todd (1920). By examining the same features originally described by Todd (i.e., dorsal plateau, ventral rampart, and symphyseal rim), they developed a scoring technique that provides a final mean age based on a composite score (McKern and Stewart 1957), rather than a phase system. In this method, the practitioner has the ability to categorize the element by stages rather than phases. Each stage (0=absence of trait; 5=presence of trait) represents the magnitude and gradual progression of the trait. Along with the pubis symphysis, McKern and Stewart also incorporated other skeletal elements into this method (e.g., cranial sutures, vertebrae, clavicle, sternum, scapula, and extremities).

Even though McKern and Stewart were able to refine Todd's original technique, inherent flaws were noted on McKern and Stewart's reference sample. The sample used to develop this technique was composed of American war casualties (males from the Korean War), with ages ranging from 17 to 50 years of age, thus skewed towards younger males. Of the 450 individuals included in this study, 78% (n=352) of the sample was between 17 to 23 years of age, with a minimal representation of older individuals.

Methodological problems with previous work by Todd, and McKern and Stewart, motivated Suchey and colleagues (Suchey-Brooks 1990; Katz and Suchey 1989; Brooks 1955) to establish a new methodology addressing the issues of sample bias, limited age range samples, lack of testing on multiple populations, and small age intervals included in each phase. Thus, the Todd ten-phase method was modified by Suchey-Brooks into six phases of aging of the pubic symphysis (Katz and Suchey 1986; Katz and Suchey 19889; Suchey et al. 1986; Brooks and Suchey 1990; Suchey and Katz 1998). The Suchey-Brooks study was based on 739 males and 273 females from a well-documented forensic sample at the Medical Examiner's Office in Los Angeles. In contrast to Todd and McKern and Stewart's technique, this method describes the average expression of age related changes in six phases, as opposed to the analysis of skeletal features by assigning numerical scores. Despite several improvements in the technique, the Suchey-Brooks method produces large overlap between the ages included in the six phases, and more importantly, is rooted in a descriptive scoring system. Furthermore, previous studies have highlighted the impression and inaccuracy of this technique when applied to individuals over the age of 40 on several populations across the world (Meindl and Lovejoy 1989; Milner and Boldsen 2012; Martrille et al. 2007; Kimmerle 2008). Over the last several decades, various attempts have been made to revise the pubic scoring system criteria and advance methodological improvements (Lottering et al. 2013; Meindl et al. 1985; Slice and Algee-Hewitt 2015; Stoyanova et al. 2015; Stoyanova et al. 2017); however, many of these new methods have been overshadowed by the simplistic nature of the Suchey-Brooks method, thus continuing to be the most widely used adult age estimation method among forensic anthropologists (Slice and Algee-Hewitt 2015).

Difficulties with the estimation of age at death

Age-at-death derived from the adult skeleton is inferred from an imperfect correlation between the biological age (skeletal expression stages) and chronological age (calendar years since birth) of an individual. Even though it is known that the correlation between these two variables is imperfect, it is accepted in biological anthropology that the age of the adult skeleton could be subjectively estimated from skeletal elements that undergo progressive morphological changes over time. Many of the techniques previously discussed in this chapter rely on a phase-to-age approach for the estimation of age. The phase-to-age approach requires the practitioner to visually examine the age informative markers (i.e., pubic symphysis, sacroiliac joint) and properly apply the descriptive changes of each phase to a score, which then needs to be correctly translated to a chronological age range. For example, for the Suchey-Brooks method, the practitioner is expected to visually determine age-related changes in the skeletal morphology of the pubic symphysis and associate those changes against a set of specific criteria represented by textbook descriptions and casts of the pubic symphysis representing a series of pre-defined age phases established by a reference sample. Difficulties with this approach for age estimation in the adult skeleton have been widely recognized (Kimmerle et al. 2008; Garvin and Passalaqua 2012). Some of the difficulties encountered when using methods that require the practitioner to assess the morphological aging features and then assign them to an age phase include; 1) inter- and intra-observer error, 2) broad age intervals (i.e., Suchey-Brooks male phase IV 23-57 years) and overlap of phases, 3) biased estimates (i.e., overestimation of younger individuals and underestimation of older individuals), 4) and age mimicry (age estimates for the target sample reflect the ages of the reference sample when inadequate priors are used).

First, with phase-to-age techniques, changes in skeletal morphology are assigned to discrete, universally fixed, ordered stages that are associated with changes in chronological age. Because these methods use phases that require the practitioner to subjectively assign the skeletal trait to one of several constructed categories, considerable intra- and interobserver error is encountered when applying the technique to an unknown sample. In this case, accuracy and reliability are dependent upon the experience of the practitioner. This issue was highlighted by Kimmerle and colleagues (2008), who investigated how consistently several investigators assign skeletal traits to phases described in the Todd's ten-phase system and the Suchey-Brooks six-phase system. Results demonstrated that significant statistical variation was observed between four observers when assigning phases to an unknown, with only two of the four observers being statistically consistent when using the Todd method. More observer variation was found when using the Suchey-Brooks method. Kimmerly and colleagues (2008) demonstrated that confusion over the descriptions of the ventral rampart formation in Suchey-Brooks phase III is problematic, providing an additional layer of interpretive errors.

Second, phase-based aging may generate wide age intervals that have little to no value for comparison with a missing person's list. For example, the Suchey Brooks phase IV for males includes ages between 23 to 57 years of age, with a mean age of 35.4 years of age. This wide age range is problematic when assigning an age to an unknown individual for the purpose of a forensic identification. Furthermore, several phases in the Suchey Brooks method tend to overlap due to the nonlinear relationship between the phase and the chronological age. For example, the Suchey Brooks Phase IV includes ages between 23 to 57 years, while the Suchey Brooks Phase V includes ages between 27 to 66 years. This overlap can confuse the practitioner when attempting to match the unknown to the best of the overlapping phases; as both phases include very similar age ranges but the text descriptions for each phase are different (i.e., phase IV: oval outline of face is usually complete but a hiatus can occur in the upper ventral rim, and phase V: face is completely rimmed with some slight depression of the face) (Katz and Suchey 1989).

Third, aside from overlapping phases, many of the well-known traditional techniques are developed using an inverse calibration, in which the dependent variable (x) is regressed against the independent variable (y). Thus, when estimating age, the independent variable of age is regressed against the skeletal indicator. When using an inverse calibration, the regression is towards the mean age of the reference sample, thus any age-at-death estimate generated will be skewed towards the direction of this mean. Masset (1989) refers to this source of error as "attraction to the middle". This is mainly due to the fact that the estimated ages closely reflect the mean age of the reference sample used to create the technique, rather than the actual chronological age of the unknown. As many of these phase-based methods are based on the regression of age on the indicator, the practitioner assumes that the unknown sample originates from a similar age distribution as the reference sample. For example, if the technique used to estimate age was created with a reference sample of individuals with a lower mean age, the technique will tend to underage older individuals and overestimate younger individuals.

Aside from "attraction to the middle", age mimicry is another problematic issue when estimating age-at-death in the adult skeleton (Hoppa 2002; Konigsberg and Frankenberg 1992). Age mimicry occurs when the age distribution of the unknown sample reflects the age distribution of the reference sample used to create the method (Konigsberg and Frankenberg 1992). Thus, the estimates created with the technique will results in an age estimate distribution that mimics the distribution of the reference sample. Several solutions to the aforementioned issues involve the use of reference samples that represent all ages and that had undergone similar extrinsic and intrinsic factors.

Unfortunately, finding or creating unbiased reference collections has been difficult to achieve. First, many of these reference collections belong to medical examiner's offices from across the world, with younger individuals being overrepresented in the collections. In contrast, reference collections at museums and universities have been developed using individuals who have died at hospitals and whose bodies are sometimes unclaimed or donated to science. Many of these skeletal collections are skewed towards older individuals of low socioeconomic status (Kemkes-Grottenthaler 2002; Usher 2002).

Aside from obtaining an appropriate skeletal reference collection sample, several studies have been attempting to address the statistical problems that affect the accuracy of adult age estimation. Transition analysis was a method published in 2002 that addresses the issues of age mimicry of the reference sample (Boldsen et al. 2002). In contrast to the traditional aging techniques, which are based on regression analysis, transition analysis utilizes Bayesian statistics to estimate adult age-at-death. Thus, transition analysis is based on the probability of an event (i.e., age-at-death) assuming knowledge of certain conditions that might be closely associated with the event (i.e., skeletal reference sample). By using Bayesian theory as the foundation for the estimation of age, the practitioner avoids the mathematical assumptions underlying the traditional aging methods, which provide replications of the age distribution of the reference sample.

The component scoring method of transition analysis (ADBOU 2002) is based on age informative markers collected from several locations of the skeleton, including the cranial sutures, the pubic symphysis, and the auricular surface of the ilium, by scoring the skeletal feature in a similar way to traditional methodology (textbook descriptions and illustrations). The scores are then inputted into a statistical software (ADBOU 2002) that creates a maximum likelihood estimate of age using prior probabilities. A validation study using a known age-at-death skeletal collection (William Bass Donated Skeletal Collection) demonstrated that transition analysis underestimates the ages of older individuals (40-70 years of age) when all three traits are used (Milner and Boldsen 2012). When the skeletal traits were tested separately, the pubic symphysis performed the best, while the cranial sutures produce the least accurate results. In a different validation study, five researchers independently applied the method of transition to analysis to a sample (n=58) of known ages from the William Bass Donated Skeletal Collection, demonstrating that the technique consistently over estimated individuals younger than 30 years of age and underestimated individual over 40 years of age (Fojas et al. 2017). Furthermore, the study demonstrated that the technique is easy to apply for an observer at any level of experience and knowledge (Fojas et al. 2017), as the majority of the traits examined during the study were in agreement between all five researchers. Although transition analysis has been a methodological advancement over traditional methods in a statistical sense, the technique still has inherent issues producing age estimates with accuracy and precision. Furthermore, the issues of intra- and inter-observer error are still very much present in this technique, as the practitioner is still expected to visually determine age-related changes in the skeletal morphology of the skeletal trait and associate those changes against a set of criteria.

Currently the component scoring approach of transition analysis is based on the pubic symphysis, sacroiliac joint, and cranial sutures. However, the authors are expanding the technique to include other skeletal elements and the capability to estimate age-at-death on younger individuals (Boldsen and Milner 2018).

Computational skeletal aging methods: Pubic symphysis

Recently, Slice, Algee-Hewitt, and Stoyanova developed three new age-at-death techniques that claim to be more objective, by eliminating textbook descriptions and casts, and statistically more robust than conventional bone-to-phase matching standards. In order to generate an age-at-death estimate, the practitioner scans the pubic symphysis using a 3D scanner, edits the scan to isolate the symphyseal face from the rest of the pubic bone, and the three-dimensional coordinates obtained from the scanning are subjected to several algorithms included in a software (forAge) provided by the authors. Once the scan is loaded into the software, a final point age estimate is obtained. According to the developers, these new techniques are expected to reduce the high degree of variation in method interpretation and low degree of standardization across application of the technique and practitioners (Slice and Algee-Hewitt 2015).

The first method, the Slice-Algee-Hewitt score (SAH), was introduced in 2015 (Slice and Algee-Hewitt 2015). It measures the variance on the symphyseal face by capturing the gradual transition from ridges and furrows (great depth) to flattening of pubic symphysis (less depth). First, standardization of the position for each scan takes place, so that the x, y, and z coordinates generated by the laser scanner match the center of a coordinate system. This is achieved by calculating the original position of the x, y, and z coordinates (i.e., finding the average values between the smallest and largest x, y, and z vertex coordinates), and multiplying the vertices, in order to center the origin (0,0,0) of the coordinate system. Thereafter, a principal component analysis (PCA) is used to rotate the scan so that the maximum variability is visible on each axis (x, y, and z). After the PCA rotation, the x-axis (largest variance; length), y-axis (second largest variance at a right angle uncorrelated to the first; width), and the z-axis (smallest variance; depth) are used to account for the spatial variation in the articular surface of the pubic symphysis. The method focuses on the change in variance of the third principal component (PC) eigenvalue (depth) generated using the three-dimensional file of the pubic symphysis. A high variance value (greater depth) is associated with younger individuals and a low variance value (little depth change) for older individuals. This method was originally created utilizing 40 males with known age-at-death from the William Bass Skeletal Donated Collection.

In 2015 and 2017, the authors established two new computational methods, the Thin-Plate-Spline/Bending Energy method (TPS/BE) (Stoyanova et al. 2015), which measures the bending energy required for transforming an infinite thin and perfectly flat plate to match the surface of the symphyseal face, and the Ventral Curvature method (VC) (Stoyanova et a. 2017), which measures the curvature of the ventral margin to capture the progressive formation of a rim in the symphyseal face.

The algorithms for the Thin-Plate-Spline/Bending Energy method (TPS/BE) measure the required energy for transforming a perfectly flat, infinitely thin plate to match the surface of the pubic symphysis scan. The minimum energy that is required for this bending is expected to account for the changes occurring in the symphyseal phase (i.e., billowed surfaces, flattening, and breakdown of symphyseal phase). In order to apply the TPS/BE algorithm, two sets of control points need to be calculated. The first set of points lies on a flat plane (i.e., calculated by a uniform squared mesh on a plane against the scan) and a second set of points that lies across the surface of the pubic symphysis (i.e., calculated by a uniform mesh that overlaps the scan morphology). The corresponding points on each mesh have the same x and y value, but different z values (depth). The points on the flat plane have a z value of 0. After the two sets of control points are calculated, the thin Plate Splines model the bending of the flat plate to match the mesh from the pubic symphysis morphology. The force needed to bend the plate is calculated as the bending energy, which is used to create an age score. After obtaining the bending energy score, a linear regression analysis produces an age estimate.

A third set of analysis involves the algorithms for the Curvature of the Ventral Margin method, which captures the progressive formation of a rim around the entire symphyseal surface and its later erosion. As in the TPS/BE method, meshes with different densities are generated to cover the entire surface of the pubic symphysis, but the points inside the symphyseal face are deleted, leaving vertices (equidistant semi-landmarks) around the outline of the face. The algorithm created by Stoyanova et al. (2016), quantifies the progressive changes associated with the outline of the pubic symphysis. The algorithm uses least squares to find the best fitting circle through the selected semi-landmarks on the ventral half of the symphyseal outline. The curvature of the ventral margin will be measured as 1/r; thus, a large radius implies a small curvature value (younger individuals), and a smaller radius produces higher curvature values (older individuals). After a score value of the ventral curvature is obtained, a linear regression analysis provides a point age estimate.

An updated version of the software, published in 2017, calculates all three algorithms separately, each providing a point age estimate. In addition, the software also incorporates multivariate regression models combining all three techniques. These new multivariate methods were established using 93 White males from the William M. Bass Donated Skeletal Collection. A validation study conducted by Stoyanova et al. (2017) demonstrated that by combining the SAH-score with the Ventral Curvature, and the Bending Energy with the Ventral curvature, in a multivariate approach, the models produce objective age-at-death estimates comparable to traditional aging techniques, like the Suchey-Brooks method. As previously stated, the reliability and reproducibility of these methods has not been evaluated in other reference samples or with a larger sample size.

The following chapter provides a preliminary understanding of the population history of Mexico and Puerto Rico, including a summary of how historical events (i.e., migration, colonization) shaped the morphological and genetic variation of each group. Furthermore, the following chapter includes a summary of the historical background of the term Hispanic in the U.S., and how it influences the field of forensic anthropology.

CHAPTER THREE
MORPHOLOGICAL AND GENETIC VARIATION: MEXICO AND PUERTO RICO

The demographic structure of the United States (U.S.) has shifted over the last decade as the result of significant immigration waves from Mexico and the Caribbean. As a result, individuals who self-identify as Hispanic or Latino are now the largest growing demographic group in the U.S, with Mexicans and Puerto Ricans accounting for more than 74% of this group (Gutierrez 2016). This will most likely result in an increase of medico-legal cases involving human remains presumed to be of Mexican or Puerto Rican ancestry. Yet, biological parameters that can aid in the positive identification of populations originating from Latin America have not been systematically evaluated against traditional techniques established with reference collections from American White and African American individuals.

One of the main goals of this study is to examine whether Mexican and Puerto Rican samples should have two different age-at-death techniques developed, or if they should be pooled in order to develop a single age-at-death method. The goal of this examination is to further answer questions related to the effects of group specific and individual life history differences in senescence and their consequences to the morphological expression of the pubic symphysis. First, in order to address the goals of this study, it is important to have a sound understanding of the population history of both samples included in this study. This chapter provides a preliminary understanding of the population history of Mexico and Puerto Rico, and how these events shaped the morphological and genetic variation of each group. For the purpose of this study, the Hispanic and/or Latino term is used throughout the following chapters as populations who reflect admixture proportions from Native American, Europeans, and/or West Africans.

Hispanic/Latinos in the United States: Population history of the Caribbean and Mexico

The Office of Management and Budget (OMB) defines Hispanic or Latino as an individual of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish cultures or origin living in the U.S. (www.census.gov), and it is designated as an ethnic category in the Census, rather than a racial category. The term Hispanic was first implemented in the U.S. Census in 1980 during the Nixon administration. In contrast, the term Latino, ending in” o” which in the Spanish language signals a male descriptor, has been traditionally used to encompass both male and female genders, whereas Latina, ending in “a”, refers exclusively to female gender. Nonetheless, in response to the gender-neutral language debate, the term has evolved within the academic literature and social media as the gender-neutral term Latinx (Salinas and Lozano 2017).

In forensic anthropology, the term Hispanic and/or Latino is widely used to describe skeletal samples that originate from a Latin American country or that could potentially reflect genetic compositions similar to populations of Latin American origin. However, the terms provide an inaccurate representation of Latin American populations, by ignoring the different ethno-histories (e.g., migrations, intermarriages) of the many populations included in this definition (Ross et al., 2004), and simplifying complex populations by lumping them into one category. It has been demonstrated by previous studies that phenotypic and genetic variations reflect the different ethno-histories from a specific population (Via et al., 2010; Martinez-

Cruzado et al., 2008; Rangel-Villalobos et al., 2008; Rubi-Castellanos et al., 2009). In other words, variations in physical appearance, including skeletal features of the adult skeleton are accompanied by specific patterns in the genetic code, or genotype. As forensic anthropologists rely upon phenotypic variation for the development of techniques for the creation of a biological profile, it is important to have scientific agreement and deep understanding of the population history of groups who are included in the Hispanic/Latino definition.

Individuals who now reside in the United States and who self-identify as Latinos or Hispanics have long and complex historical lineages rooted in the territorial and economic expansion of the United States (Gutierrez 2016). According to Gutierrez (2016), *"the territorial conquest was the first force that incorporated Latinos into a polity" (*p.96). Many Latinos/Hispanics entered the United States willingly as immigrants in the 20th century due in part to the Bracero Program and the Gold Rush; however, many were territorially incorporated as the result of wars (i.e., U.S.-Mexican War in 1848 and the Spanish American War of 1898) and the American imperial expansion that started in the 19th century (Guitierrez 2016; Engerman 2000). In order to understand the evolution of Hispanic/Latinos in the United States, we must first understand the history of imperial concentration in the Americas, beginning in 1492 with Christopher Columbus.

The first era of Spanish imperial settlement in the Americas was in the Caribbean, with Puerto Rico, Cuba, and the Hispañiola as its principle sites of settlement (Engerman 2000; Guitierrez 2016). Estimates of indigenous populations in the Caribbean vary from 500,000 to almost 10 million before European colonization. There is uncertainty about the dates when the indigenous groups arrived in the Caribbean; however, many researchers agree that the main three groups belong to three different but similar cultures: Ciboney, Arawak, and the Caribs (Curet 2005; Engerman 2000).

After European colonization, the Caribbean experienced a demographic shift in population due to the arrival of many European migrants, and thereafter, with the transatlantic slave trade. Spaniards settled in Cuba, Puerto Rico and Dominican Republic, while the French settled in Haiti thereafter. According to historical records, early during the Spanish conquest (1492-1620) the indigenous populations were decimated from the entire Caribbean, due to warfare, diseases, and forced labor (Guitierrez 2016, Engerman 2000; Curet 2005). Archeological studies conducted at the *Paso del Indio* in the northern region of the island of Puerto Rico, have shown evidence of syphilis on archeological skeletal remains (Curet 2005; Crespo-Torres 2005). It is believed that most of the indigenous groups that survived the introduction of diseases and war conflict by Europeans were forced to work in sugar cane plantations, introduced into Catholicism, and obligated to assimilate a more European way of life (Engermann 2000; Curet 2005).

In the 14th and 15th century, enslaved Africans were sent to Southern Europe, but by the 16th century the development of the transatlantic slave trade began to the Caribbean (Engermann 2000). In response to the declining of Native populations, King Charles V of Spain granted permission to export the first group of enslaved indigenous Africans (4,000) to serve as the primary laborers in the Antilles by the beginning of 1518. The expansion of sugar production created a need for a new labor force, giving rise to African slave trade. As a result, more than 90% of the population in Cuba, Puerto Rico, and the Hispañiola consisted of enslaved Africans, and Europeans represented only 10 percent (Curet 2005; Engerman 2000). The proportion of enslaved Africans to Europeans was different in islands in the Caribbean that did not have sugar production,

The slave trade continued for 2 centuries and ended in 1860. It is believed the Caribbean received more than two fifths of the entire slave populations that were transferred to the Americas (e.g., 10 million). From these numbers it is believed that about 60 percent of the enslaved African population was located in the Greater Antilles (e.g., Puerto Rico, Cuba, The Hispañiola), and the rest was located in the British and French islands. It is also worth noting that the enslaved population consisted of mainly males (Guitierrez 2016, Engerman 2000; Curet 2005).

In the early 16th century, Spaniards subsequently conquered the Aztecs in Valle of Mexico City. This area had a dense indigenous population that supplied most of the labor under Spanish rule, and only a few enslaved Africans were imported to this area. Similar to Mexico, much of the island of Puerto Rico was dominated by Spaniards, who directed most of the sugar plantations in the northern and southern regions of the island. Due to the high demand for sugar production, European migration (e.g., Irish, Dutch, Portuguese, French, among others) increased in Puerto Rico. Sugar plantations were established in the southern and northern regions of Puerto Rico, whereas many others established coffee and tobacco haciendas in the central regions of the island. European migrants were considered to be of a higher socioeconomic status within the population, while some creoles (Europeans and Indigenous mix) had only access to a few plantations/haciendas, making them part of the working class (Engermann 2000; Sacarano 1984). The lower strata of the population were considered to be the enslaved Africans working on the haciendas and plantations. After the abolition of slavery in the late 1800's, many African natives could not return to their homeland and therefore stayed on the island still working in haciendas and plantations, but as paid workers (Sacarano 1984).

When the US-Mexico War ended in 1848, the United States took nearly half of Mexico's national territory. Mexican citizens residing in the annexed territory were given one year to move back to Mexico. Approximately 100,000 Mexicans chose to stay, and within a year were granted American citizenship (Gutierrez 2016). In the Caribbean, by 1898 Spain lost control of Cuba, Guam, the Philippines and Puerto Rico, and several years later Puerto Rico became a territory of the United States. The change in sovereignty (US occupation) resulted in a complete reorganization of the agrarian economy, and by the 1870s the sugar and coffee industries were declining, and manufacturing and services economy replaced many haciendas. However, the island of Puerto Rico experienced a rise in unemployment, which ultimately increased emigration to the United States by the beginning of the 1970's.

Similar to Puerto Rico, during the late 18th century, the Mexican population was experiencing extreme levels of poverty and several famines (e.g., 1786-1787 "El año del hambre" was the worst famine) which resulted in an increase in the percentage of diseases across the country (Mccaa 2000). These events created a baseline to what today is known as one of the worst centuries in Mexico, the 19th century, which promoted a decline in population growth, high mortality rates and prevalence of infectious and gastrointestinal diseases (López-Alonso 2012; Mccaa 2000).

During the beginning of the 19th century, the Mexican elite were determined to promote European migration to the country in order to emulate the economic and political growth of the United States (López-Alonso 2012; Mccaa 2000). It is worth noting that Mexico did comparably well in its economic and demographic growth after independence in 1821, and some accounts compare this economic growth with that of the United States. However, a gap started to become more apparent once the Mexican government (e.g., with the influence of Mexican Elite) began to implement anticlerical policies and eliminate governmental aid for the poor (López-Alonso 2012; Mccaa 2000). Many Mexican elites believed the Indigenous populations across the country were an obstacle for Mexico's economic and political progress, and that having such a high percentage of Indigenous and poor populations in Mexico would make the country less attractive to European migrants (López-Alonso 2012; Mccaa 2000). For this reason, the Mexican government instituted anticlerical campaigns against the church and any other organizations that provided aid to indigenous populations and low-income families. Many believed that these two groups were less fit for survival, thus there was no logical reason to help populations that couldn't be saved (López-Alonso 2012; Mccaa 2000). These government campaigns had a tremendous effect in the lower-class sector of Mexico, with previous census records showing that the country experienced a 5% decline in population growth, mortality rates increased, and diseases became more prevalent (Mccaa 2000). Based on military records from the federal and rural militia, many of the young individuals with birth years ranging between 1840's to 1850's presented a decline in stature when compared with Pre-Hispanic (archaeological) samples from Tehuacán, Teotihuacan and Oaxaca (López-Alonso 2012). This decline in stature provides evidence of the deterioration of the well-being and living standards of many groups across Mexico. It is worth mentioning that these stature estimates belonged to individuals living in rural and metropolitan areas across Mexico, thus reinforcing the notion that the whole country was experiencing a decline in its standards of living at the time.

Nearing the end of the 19th century, the Porfirio Díaz administration took control of the economic and political opportunities of the country. This administration promoted economic growth, modernization and political stability to the country. A major change that occurred during this period was the creation of charitable organizations that helped the lower class; however, these centers were limited and only available in the metropolitan areas of the country, like Mexico City (e.g., it is estimated that only three to four charitable societies were created in Mexico City).

Two other main events that took place during the Díaz administration were the appropriation of land from Indigenous groups across the country, and the increased wage gap between the northern and southern regions of Mexico (López-Alonso 2012; Mccaa 2000). It was still a pressing matter for the Diaz administration and the Mexican elite to promote European migration, and for this reason, the government seized many of the indigenous properties across the county to make them available for Europeans to establish plantations and haciendas. It was of special interest for the government to seize land located in the southern regions of Mexico, known for their good climate and fertile agricultural grounds. However, by the end of the 19th century, the government realized that Europeans were not migrating to Mexico as expected, and by this time period 95 percent of the population in Mexico was mainly composed of Mestizos (e.g., Indigenous, European and African ancestry). The appropriation of land promoted a decimation of Indigenous groups across the southern regions of Mexico, thus promoting their steep decline and an increase in mortality rates (e.g., infectious diseases). Much of the land seized was then used for plantations and haciendas controlled by the Mexican elite, and many indigenous groups were relocated to work on those plantations and haciendas.

The Diaz administration was interested in promoting modernization and transportation routes along the Northern regions of Mexico and the United States. While in the southern regions the government promoted agriculture with the implementation of haciendas and plantations, the northern and central regions of Mexico were considered to be the epicenter for growth and development of the country. Some states in the *bajío* region (e.g., Hidalgo, Veracruz) were increasing their economic development with mining opportunities in Hidalgo and the creation of ports around the Veracruz coast for exportation and importation of material goods, while the northern regions were expected to grow with the creation of railroads and material exports to the United States (Lopez Alonso 2012). Due to the economic success of these regions, the government expended all its energy and capital in the northern region, especially those areas close to the border with the United States. This government decision created an immense wage gap between the northern and southern regions of Mexico. In contrast to the southern regions, the wage average for northern workers was from a high of .83 cents a day to .75 cents a day. This also created an increase in internal migration, with many individuals leaving the southern rural regions of Mexico to seek better economic gain in the north. As a result, the southern region of Mexico not only experienced a decimation of Native Indigenous groups, but also experienced an overall population decline due to the economic developments of the north.

Previous studies conducted by historians have been able to portray how the events that took place during the 19th century affected the social well-being of the Mexican population, especially the lower and working class of the country (Lopez-Alonso 2012; Mccaa 2000; Katz 1974). However, demographic data for this period is limited (e.g., income per capita, and mortality and birthrates) making it challenging for researchers to accurately examine the social events of the Diaz administration. Many researchers have relied on stature information from military records to examine the living standards and socioeconomic development of the century (Lopez-Alonso 2012; Mccaa 2000; Katz 1974). Lopez-Alonzo demonstrates that the stature of the Mexican lower class declined during the Porfirio Diaz administration by comparing stature estimates from a Mexican group that at the time was considered to be the elite (e.g., individuals who applied for passports to travel out of the country) with military records of young males in Mexico. Lopez-Alonzo demonstrates that the stature of the elite group increased 4 cm during the time period Porfirio Diaz took over. It is worth noting that the elite sample experienced an increase in stature at the same rate that the income per capital increased in Mexico City. Lopez-Alonso (2012) also includes in her analysis a sample of passports of individuals considered to be part of the working class in Mexico City (e.g., nannies from the elite) who also present an increase in stature in comparison with the military sample during the Porfirio Diaz administration.

In response to the negative effects of the Diaz administration in the lower and working class of the population, the Mexican Revolution took place during the beginnings of the 20th century (1910-1920). After the Mexican Revolution ended, the Cardenas administration took control over the country. It was during this time period called the "*Mexican Milagro*" (e.g., Mexican Miracle) that the government implemented charities for the poor in the rural areas of the country, including vaccinations programs, better health care programs and overall better standards of living across the country. Nevertheless, the impacts of the Diaz administration and the Mexican Revolution took a toll on the economic and political development of the country.

Due to the economic hardship many Mexicans encountered during the 19th century, migration to the United States increased. Many historians attribute the increase of migratory movements to the United States to the development of the Bracero Program. During WWII, the United States government feared that a shortage of laborers in the United States would impact the agricultural economy (Snoodgrass 2011). Thus, the Mexican and United States government implemented the Bracero program in the early 1920s. The program contracted guest Mexican labor for a specific amount of time (e.g., 1, 3, or 6 months), and the workers would provide their services to farms across the United States. The farm owners would have to provide housing, food, adequate pay, and training to the workers in order to participate in the program. It also included a savings program, in which 10% of their salary would be saved until the end of the workers' contract. The program gained popularity in Mexico and by the first year close to 50,000 workers had contracts with multiple farms across the United States. By the end of the third year, applications to the program had nearly tripled. The increase in salary in comparison with Mexican farms made it possible for many families to use the Bracero program as their only source of income (Snodgrass 2011). Many believed that the program would not only provide monetary gain, but also a future for the Mexican agricultural economy. It was expected for many of these workers to gain advanced training in the United States, so that later these workers would return to Mexico and utilize those skills in Mexican soil. However, many Mexican workers stayed in the United States (documented and undocumented), thus increasing the migratory shift of the population. The Bracero program lasted 20 years, but the United States did not renew the contract with Mexico since undocumented Mexican workers already covered much of the labor demand in US farms. It is believed the Bracero Program is one of the main factors that promoted undocumented border crossing to the U.S.

In summary, the period of colonization across the Caribbean and Mexico provides a better understanding on how the historical events affected many Hispanics or Latinos. Colonization gave way *"for ethnic or national identities to be forged historically, sometimes of one's own making, but, just as often, imposed from above by dominant groups and thus either accepted or resisted at the personal level" (Gutierrez 2016, p96).* The aforementioned overview of population history gives way to a relevant discussion in our efforts to understand the genetic and skeletal variation within Mexico and Puerto Rico.

Genetic and skeletal variation within Mexico and Puerto Rico

Hispanic and/or Latino groups exhibit complex population structures, with significant genetic ancestral contributions from Europeans, Native Americans, and West Africans (Gonzalez Buchard et al. 2005; Simms et al. 2010; Rubi-Castellanos et al. 2009). Even though most forensic anthropologists do not examine genetic data in the creation of a biological profile consisting of estimation of age, sex, height and ancestry, understanding geographical trends of variation from both genetic and skeletal data allows for an integrative understanding of the phenotypic expression of the population in question. Traditionally, when contributing to an individual's biological profile, forensic anthropologists have relied upon different techniques, including metric and non-metric traits, that delineate the skeletal attributes common to a particular ancestral group (population specific methods). The quality of the results produced by these techniques depends on the anthropologist's choice of the most appropriate population against which to classify the unknown, and properly employing the technique (Algee-Hewitt

2013). Most of the current techniques employed by forensic anthropologists assume a direct correlation between the morphological characteristics examined and the genotype that is driving the expression; however, most forensic anthropologists do not acknowledge this assumption. Inadequate understanding of this assumption can lead to the use of inappropriate methodology, and therefore harm the chances of identification.

For example, Spradley and colleagues (2008) presented evidence that Hispanic male crania were misclassified 60% of the time as female when using discriminant function analysis for the estimation of ancestry. Spradley et al. (2008) results demonstrated that Hispanic crania and postcrania are often misclassified by forensic anthropologists because of their small size and more gracile nature than other groups such as American Blacks and Whites. Cranial morphology has a strong heritability component (inherited genetic factors); therefore, Spradley and colleagues (2008) recommend not only an analysis of skeletal morphology, but also an understanding of how the genetic component is driving the phenotypic expression, before attempting to develop new methods for Hispanic groups.

Skeletal morphology is often assumed to mirror patterns of genetic variation to a certain degree (Relethford, 2004; Relethford and Harpending, 1994). Comparisons of molecular data (Short Tandem Repeats (STR), Short Nucleotide Polymorphism (STR)) and craniometrics have indicated that cranial differences generally resemble molecular patterns within populations. Furthermore, studies examining global patterns of cranial variation via geometric morphometric analysis, have shown a close correspondence between phenotype and geographic distance across populations (Relethford, 2004; Relethford and Harpending, 1994). However, this is under models assuming neutral expectations of evolution like cranial morphology (Relethford, 2004; Relethford and Harpending, 1994; Roseman 2012).

For example, trends in craniometric variation have been shown to present similar patterns to those obtained from DNA markers at a localized level. Hughes et al. (2013) examined whether craniometric variation among contemporary Mexicans is comparable to what was reported in previous genetic studies. Results from her study demonstrated that cranial morphological sample data clusters were similar to the regional groupings inferred from genetic data in Mexico. Hughes et al. (2013) provided evidence of a gradient in the population structure of her sample, with Amerindian ancestry proportions increasing from North to South, while conversely, European ancestry proportions estimates increasing from South to North. Algee-Hewitt and colleagues (2018) were able to confirm the results of Hughes et al. (2013) and Hughes et al. (2017), by examining craniometric data from 113 individuals labeled as Hispanic in the Forensic Data Bank. Results suggested that cranial morphological variation validates the trends reported in genetic studies, with a distribution of Native American ancestry in a northwest to southeast geographic cline (Algee-Hewitt et al. 2018).

Herrera and Spradley (2012) were able to demonstrate that biological distance matrices derived from craniometric and mitochondrial haplogroup frequencies among three Mexican populations were statistically significantly correlated. Slice and Ross (2004) provided evidence showing that a contemporary skeletal sample (craniometrics) from Mexico clustered more closely with Amerindian genetic ancestry, than European and African ancestry. Martinez-Abadias and colleagues (2006) also presented evidence that cranial morphology from central

Mexico reflects the temporal component of genetic variation, which is related to the historical events of colonialism and migration around the area.

The aforementioned studies highlight the notion that human biological variation can be geographically structured. However, it can also be inferred that certain populations across the world can be group closer together than others. If suggesting that certain population groups can be group closer together than others, should skeletal techniques follow this genetic trend? Studies in population genetics might provide a potential answer to the understanding of population specific techniques within the field of forensic anthropology. Previous studies have suggested that at a global scale, there is at least evidence for five genetic clusters, which correspond to broad continental regions (Novembre et al. 2009; Ramachandran et al. 2005). Several biological explanations for the formation of these clusters include (1) the model of isolation by distance, in which the amount of gene flow between two populations decreases as a function of distance, (2) a long history of restricted gene flow (lack of migration between populations and no genetic material is being transferred), (3) and secondary contact between different ancestral populations, where two populations that no longer exchange individuals begin to exchange individuals with one another (Novembre et al. 2009; Relethford 2012; Ramachandran et al. 2005).

One of the most extensive studies conducted examining how meaningful genetic clusters are in a worldwide sample, was performed by Rosenberg and colleagues (2002). By examining approximately 1,000 individuals from 52 geographical populations and molecular data (STR) associated to each population. Results from this study suggested that individuals could be partitioned into six main genetic clusters: Africa, South Asia, East Asia, Europe, Oceania, and the Americas. Sub-clusters that corresponded to individual populations were also identified, thus providing evidence of a clinal distribution.

In addition to continental clusters, previous studies have suggested a clinal distribution of allele frequencies (Rosengber 2002; Serre and Paabo 2004). Similar to skeletal data, distributions in allele frequency allow a similarly geographically structured distribution. Studies have demonstrated that clinal distributions of allele frequencies have been the result of spatial arrangements that occurred as humans expanded from Africa (Novembre 2002). Furthermore, studies examining the relationship between genetic variation and the expansion of Africa show a gradual decrease in genetic variation (less heterogeneity) from Sub-Saharan Africa. It is believed that the expansion out of Africa produced the “allele surfing phenomenon”, in which a set of alleles from a founder population is later subdivided into different populations. Alleles that are at a low frequency in the founder population (e.g., ancestral population) will be at a higher frequency in the new colonized populations. Thus, this phenomenon is believed to produced clinal patterns in allele frequencies by producing new and gradual geographic patterns.

Part of the aims of this study is to compare several groups originating from Latin America, thus the following sections on this chapter shifts the theoretical approach from a global perspective to the two geographic areas under study: Mexico and Puerto Rico. A review of molecular and skeletal variation research is presented for a better understanding of the variation between and within these two groups.

Mexico

Mexico is considered to be a tri-hybrid population with a mixture of Indigenous groups, European and African ancestries. Previous studies have shown that the Mexican population can be stratified into different subgroups, depending on the unit of genetic analysis (e.g., STR loci, Y-chromosome). For example, Rubi-Castellanos and colleagues (2009) found that admixture estimations derived from CODIS-STR demonstrate genetic heterogeneity, displaying an increasing North to South gradient of Amerindian ancestry, and vice versa regarding European ancestry. Rangel-Villalobos and colleagues (2010) found similar results when examining Y-linked markers and concluded that significant heterogeneity between Mexican samples was present. The authors indicate that higher European ancestry is suggested in northern and western Mexican populations in contrast to central and southeastern populations. Nevertheless, other studies allude to the Mexican population as a bi-hybrid group, with a mixture from Indigenous groups and European ancestries. Bonilla et al. (2005) examined autosomal, Y-chromosome and mitochondrial ancestry informative markers (AIMs) in a group of individuals from the state of Guerrero in Mexico and concluded that the population of Guerrero has la significant lower contribution of European ancestry, and almost no contribution of West African ancestries. Guardado-Estrada et al. (2008), found similar evidence that Mexican populations arose principally from the admixture of Native Americans and European ancestries, with less contributions of West-African ancestry.

Most of the previous studies examining morphological data in Mexico depended on military, slave, or prisoner records, and little is known about how these individuals were measured (Carson 2008; Lopez-Alonso 2012). Additionally, these types of morphological data are usually available for specific time periods and for certain population groups. Lopez-Alonso (2012) found regional differences in stature between the northern, central, and southern regions of Mexico. Lopez-Alonso (2012) analyzed recruitment records of the Mexican rural and federal militia to provide new insights into the secular trends of stature of the Mexican laboring classes. Previous evidence has also suggested that craniofacial differentiation exists across Mexico. Figueroa-Soto and Spradley (2013) suggested that the southern cranial morphology, especially cranial vault, differentiates the most from the northern and central samples.

Puerto Rico

The beginning of the European colonization and transatlantic slave trade created significant changes to the population structure of Puerto Rico. In contrast to Mexico, few studies have examined the genetic admixture on the island of Puerto Rico. One of the most extensive studies was conducted by Martinez-Cruzado and colleagues (2009), who examined the mitochondrial DNA (maternal lineage) of 800 individuals on the island of Puerto Rico. Results revealed that maternal ancestries in the population are highly admixed, with 61.3% Amerindian (Native American), 27.2% sub-Saharan African and 11.5% West Eurasian. A more recent examination of the geographic distribution of genetic admixture using SNPs data on the island provided evidence that there is significant geographic variation of ancestry across the island, with African ancestry especially decreasing from East to West. Native American ancestry proportions showed reduced variation across the island and between individuals (Via et al. 2010).

Not many studies have been conducted examining the morphological variation of contemporary populations within the island of Puerto Rico as well. However, some studies have been able to examine cranial morphology of pre-contact individuals from Puerto Rico. Ross (2004) was able to examine archaeological material of pre-contact Taino groups from Puerto Rico, Cuba, Jamaica, and Hispaniola. Ross (2004) did not found significant differences between the Caribbean Tainos, excluding Cuba, suggesting some source of population influx in the

Caribbean. The lack of studies regarding the examination of morphological traits in modern Puerto Ricans is mostly due to the lack of skeletal materials available to researchers. The data collected from this study will contribute not only to the proposed study, but also to future researchers that wish to examine the variation between and within groups in Puerto Rico.

To summarize this chapter, several studies within the field of anthropology and population genetics have shown how populations from Latin American are morphologically and genetically different. Even though many of these groups still maintain shared genetic markers with complex admixture of ancestral populations, it has been shown that some of these groups can be differentiated by their unique admixture patterns. The goal of this chapter was to provide a valid theoretical justification to reflect on whether we might need to develop population specific methods, at least for samples originating from Latin America.

CHAPTER FOUR
MATERIALS AND METHODS

The goal of this study is to test the accuracy and reliability of traditional (i.e., Suchey-Brooks method) and computational (i.e., SAH score method, Bending Energy, and Ventral Curvature method) age-at-death methods when applied to several samples from Latin America, which are commonly referred to as Hispanic in the United States (U.S.). The chapter includes a description of the samples utilized in the study and an overview of data collection protocols. In addition, a detailed explanation of methods designed to test accuracy and reliability between aging methods to establish new equations for new aging models are also discussed at the end of the chapter.

Skeletal samples

In order to determine the accuracy and reliability of traditional and computational age-at death methods on Hispanic groups, two skeletal samples from two geographical regions in Latin America, Mexico and Puerto Rico, were used. These collections serve as an ideal sample to test the two hypotheses previously outlined. First, these two groups are considered to account for 65% of the Hispanic groups in the US. Thus, the sample accounts for the demographic changes occurring in the US and represents the increasing load of medico-legal cases of Hispanic background. Second, each group presents complex population structures with different ancestral backgrounds (Moreno-Estrada et al. 2013,2014; Rangel-Villalobos et al. 2008; Salas et al. 2005; Torroni et al. 1995; Via et al. 2011; Wang et al. 2008) and environmental factors affecting each group differently (Aguilera et al. 210; Carson 2008; Meaney 2010; Little and Manila 1986; Lopez-Alonzo 2012), accounting for potential differences affecting the age progression of each sample. Skeletal collections from Latin America are scarce, thus these two rare skeletal collections will help to fill the void in literature on skeletal aging in populations from Latin America. Furthermore, few Latin American samples have been used to quantify bias and error for traditional methods and not yet on computational methods (Valencia Caballero et al. 2015).

The first sample from Mexico belongs to the *Universidad Nacional Autónoma de Mexico, School of Medicine*, and represents a local population whose birth years range from mid-20th to late 20th century (n=21). This skeletal collection originates from medical examiner's cases whose bodies were donated to the Medical Examiner's Office for the sole purpose of medical education and research. The majority of these individuals were first used for educational purposes at the School of Medicine, and thereafter, used for research. All individuals included in this study died under the jurisdiction of the Medical Examiner's Office in Mexico City. In contrast to the U.S. where race (e.g., White, Black, Hispanic, Asian) and place of birth are included in the death certificate, in Mexico these options are not available in the death certificate. On a death certificate the individual can be categorized as "Mexican national", "other", or "ignore category". The physician completing the death certificate can choose any of these three options at the time of the signing of the death certificate. In Mexico the death certificate does not include place of birth. Thus, individuals with death certificates who stated that the individual was "Mexican national" where included in the study.

The second sample is housed at the *Instituto de Ciencias Forenses* in San Juan, Puerto Rico (n=60). This skeletal collection originates from medical examiner's cases whose bodies were donated to further medical education and research. Similar to the Mexican sample, these individuals represent a local population with birth years from mid-20th to the beginning of the

21st century. Similar to Mexico, the death certificate in Puerto Rico does not include an option for race. In contrast, the death certificate includes an option for the physician to write in the decedent's "citizenship at the time of death". In addition, the physician must include the place of birth, and place of death of the decedent. Individuals who were categorized as having a Puerto Rican citizenship at the time of death were included in this analysis. Due to the small sample size of females available in each collection, only male individuals are included in the study.

Data was collected from both left and right sides on all samples with documented chronological age and known sex. As in previous studies examining age-at-death (Lottering et al 2013; Schmitt 2004; Sinha and Gupta 1995; Overbury et al. 2009), only the left side was examined when testing accuracy and reliability. Left and right sides were only examined together in order to test for asymmetry. Selection criteria included the completeness of the symphyseal face and absence of pathological conditions in the pelvis. Since changes associated with aging of the pubic symphysis are due to secondary ossification that begins at approximately 18-20 years of age, the minimum age of inclusion in the project was 18 years of age.

After testing for accuracy and reliability of each sample, several statistical analyses were performed to test for group differences in aging progression. The goal of this analysis was to determine if group-specific or universal equations were needed when examining groups from Latin America. For this analysis, a third skeletal sample from Spain, considered to be a parental population, was included in the analysis. Even though it is known that Latin American samples display ancestral contributions from European, Native American, and West Africans, the largest genetic contribution for most Latin American groups is represented by individuals from Europe, predominantly from the Iberian Peninsula (Isabel Mendizabal et al. 2008; Lalaueza-Fox et al.

2001; Salas et al. 2005; Torroni et al. 1995; Via et al. 2011). Moreover, previous genetic studies have shown that Puerto Ricans and Mexican groups display genetic affinities closely related to populations specifically from Spain (Moreno-Estrada et al. 2013,2014; Rangel-Villalobos et al. 2008; Wang et al. 2008). For this reason, a skeletal sample sourced from the Medical Examiner's Office in Granada, Spain was included in the testing of group differences (n=174). This sample represents a local population from Granada with birth years ranging from mid 20th century to late 20st century. Because the main scope of the current study is to analyze the accuracy and reliability of current age at death methods on samples from Latin America only, the Spanish sample was only utilized for the analysis of group differences in age estimation and not for accuracy and reliability of methods.

The age distribution of all three skeletal samples are represented in Figure 4-1 and Table 4-1, which includes the sample size and age distribution of each sample. A total of 255 individuals were used in this study, with a mean age of 42.56 years of age. When the sample is divided by populations, the Mexican sample has a higher mean age of 53. In contrast, the Puerto Rican and Spanish sample have a similar mean age of 39.87 and 42.22 year of age, respectively. It would be ideal to have a sample that represents all age cohorts appropriately, however in this study that is not the case. Due to the nature of the sample (medical examiner's cases), younger individuals are heavily represented. However, it is important to acknowledge that the sample size from this study is larger and more ages are represented than in the original reference sample for the computational methods developed by Slice and Algee-Hewitt.

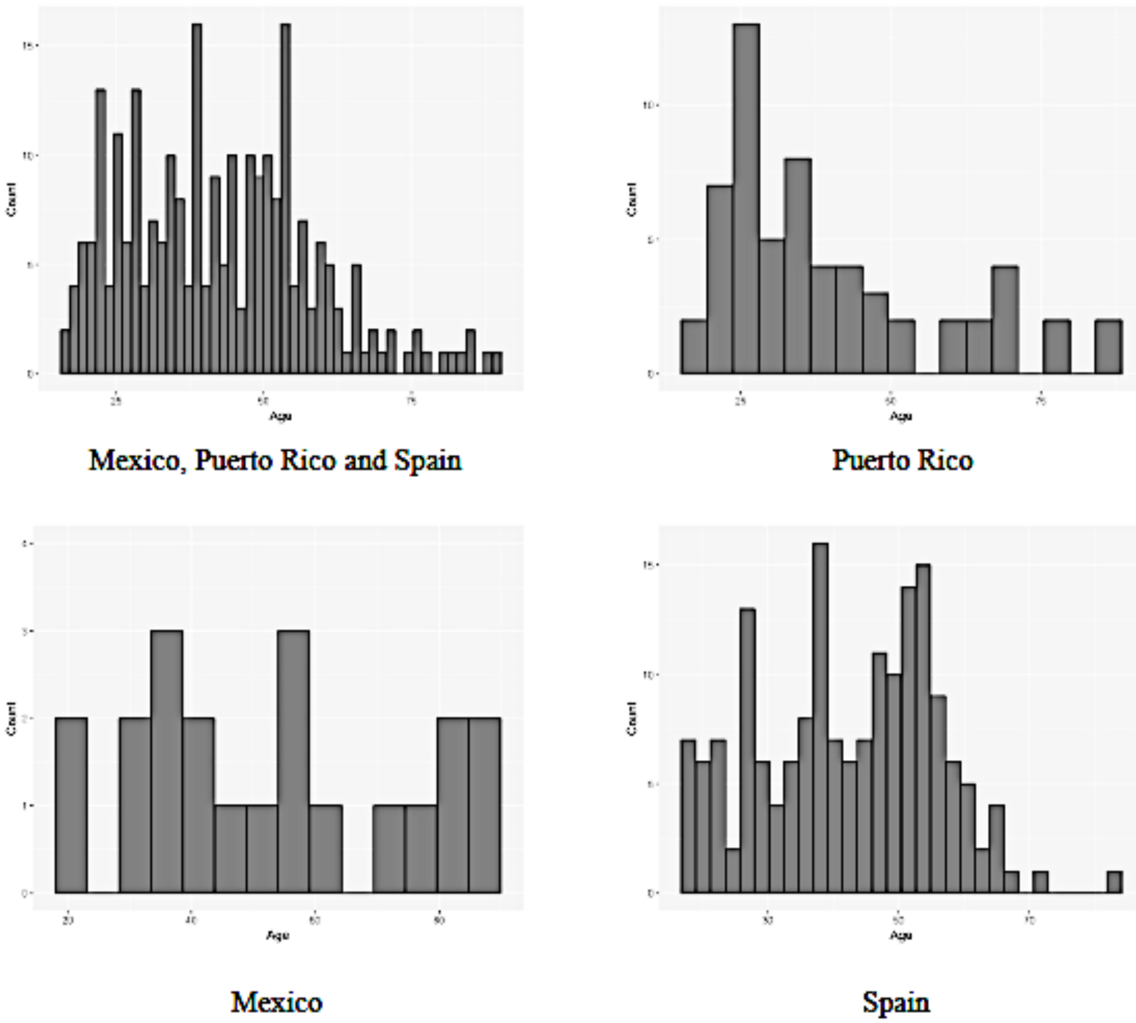

Figure 4-1. Age distribution for the pooled sample (Mexico, Puerto Rico and Spain) and each group separately.

Table 4-1Descriptive statistics for all samples

Sample	n	Min Age	Median Age	Mean Age	Max Age
Pooled (Mexico, Puerto Rico, Spain)	255	18	41	42.56	90
Mexico	21	18	52	53	90
Puerto Rico	60	19	33.50	39.87	88
Spain	174	18	43	42.22	82

Data collection

The Suchey-Brooks (1990) age-at-death estimation method was applied to the Puerto Rican and Mexican samples in order to test for accuracy and reliability of estimated ages within samples. Only the left side was scored. The Spanish sample was not available to score at the time of this study due to travel and time constrains. Each pubic symphysis was assigned to one of six phases based on characteristics involving ridge development and furrows, development and degeneration of the ventral and dorsal aspect of the symphyseal face, presence of lipping, and signs of erosion (Brooks and Suchey 1990). After each individual was scored, a point age estimate (the mean per phase) and the range (95% range) were assigned to each individual using the published guidelines.

To generate the data needed to calculate the three shape measures proposed in the Slice-Algee-Hewitt score (SAH Score) method (Slice and Algee-Hewitt 2015), the Thin-Plate Splines/Bending Energy method (BE) (Stoyanova et al. 2015), and the Ventral Curvature method (VC) (Stoyanova et al. 2017), 3D laser scans were taken for both sides of the pubic symphysis on the Mexican, Puerto Rican, and Spanish samples. For each pubic symphysis, 3D scans were created using the NextEngine 3D Desktop Scanner, 2020i (NextEngine, Inc. 2000). Each pubic bone was positioned between 7.5 and 9.5 inches away from the scanner box with the symphyseal face being perpendicular to the base of the AutoDrive scanning stand (Figure 4-2). Oil free clay was used to stabilize the bone on the stand. Scan settings were established by using the high definition scanning setting (i.e., a neutral image capture of 16 divisions, and 67K points per square inch). After each scan, data manipulation (auto alignment and fusion) took place using the accompanying software, Scan Studio HD version 1.3.2 (NextEngine, Inc. 2000). The mesh of the

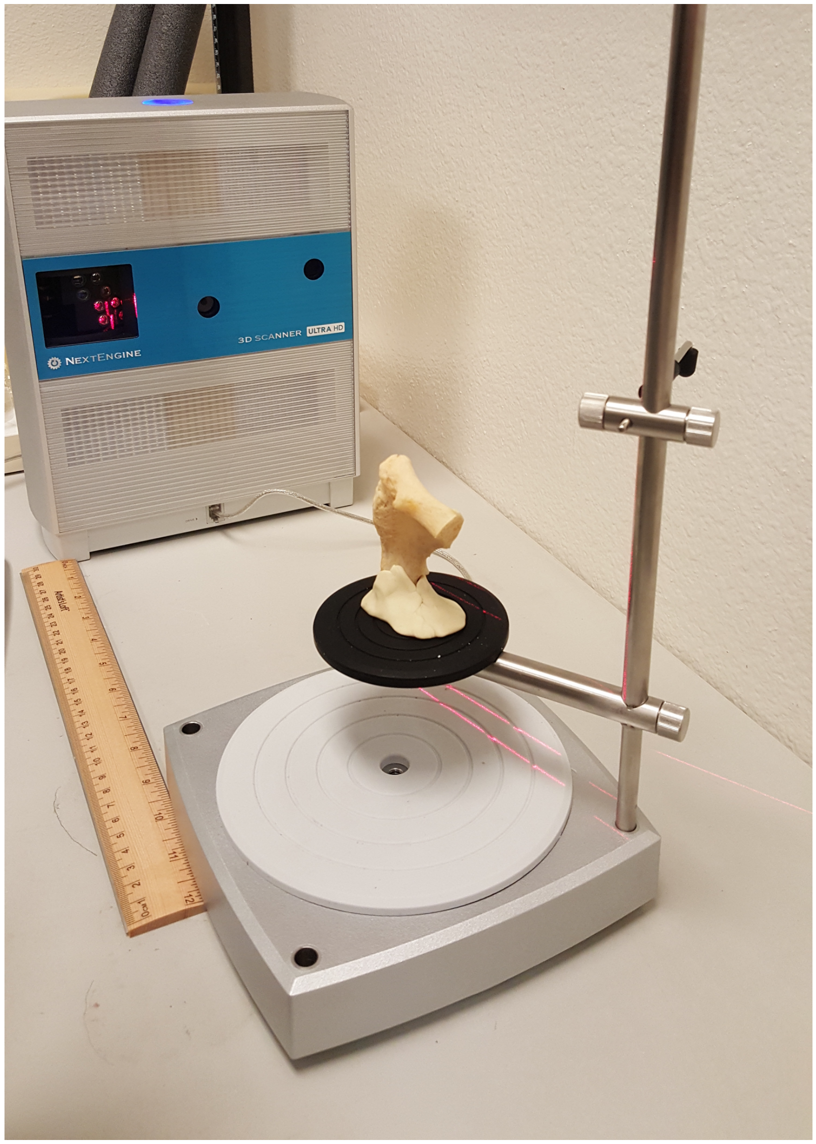

Figure 4-2. Each pubic symphysis was positioned 9 ½ inches away from the scanner box.

symphyseal surface was isolated from the rest of the pubic bone and the surrounding areas of the bone were deleted (tubercle, body of the inferior ramus, pubic body) in order to create a multidimensional model of the pubic symphysis (Figure 4-3).

Each 3D model was saved as PLY files, which consist of two lists of numbers, one including the x, y, and z coordinates and a second list of information on how the vertices are connected. Each PLY file was uploaded in a multiplatform, open source software created by the authors (Slice and Algee-Hewitt 2015), *forAge* (http://morphlab.sc.fsu.edu), in order to generate an age-at-death value using the built-in reference sample of documented White American males (Figure 4-4). As of today, this is the only reference sample available in the *forAge* software.The algorithms included in the *forAge* software calculate three shape scores used to estimate age-at-death via regression analysis following the protocols for the SAH Score, the Thin-Plate Splines/Bending Energy Score, and the Ventral Curvature Score. The SAH Score (Slice and Algee-Hewitt 2015) follows a variance-based approach by subjecting the scan vertices to a principal component analysis. In this method, the third principal component (depth of the symphyseal face) represents the age-progressive transition of the symphyseal face from ridges and furrows towards flattening. The first principal component measures the length of the symphyseal face and the second principal component measures the width of the symphyseal face. As the SAH Score algorithm captures the change in variance of the third principal component (PC) eigenvalue, a high score variance value is associated with younger individuals and a low variance value associated with older individuals.

The second score provided in the *forAge* software is for the Thin-Plate Splines/Bending energy (TPS/BE) method (Stoyanova et al. 2015). The goal of this method is to measure the

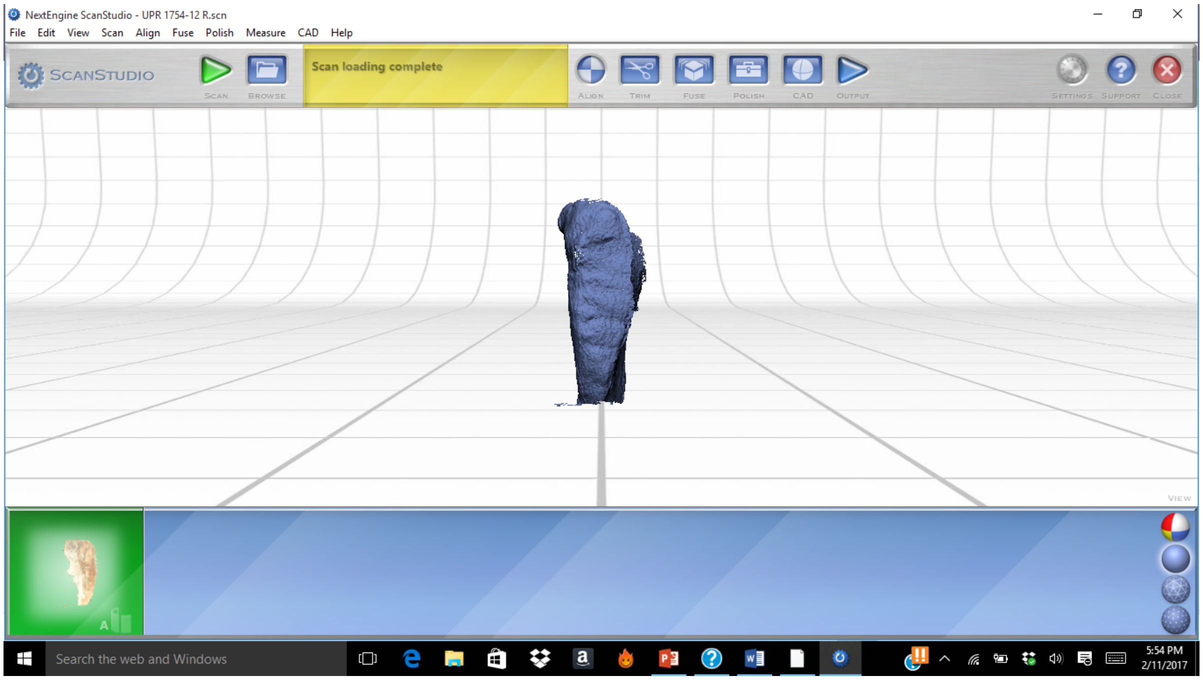

Figure 4-3. After each scan, data manipulation (auto alignment and fusion) took place using the Scan Studio HD version 1.3.2 software.

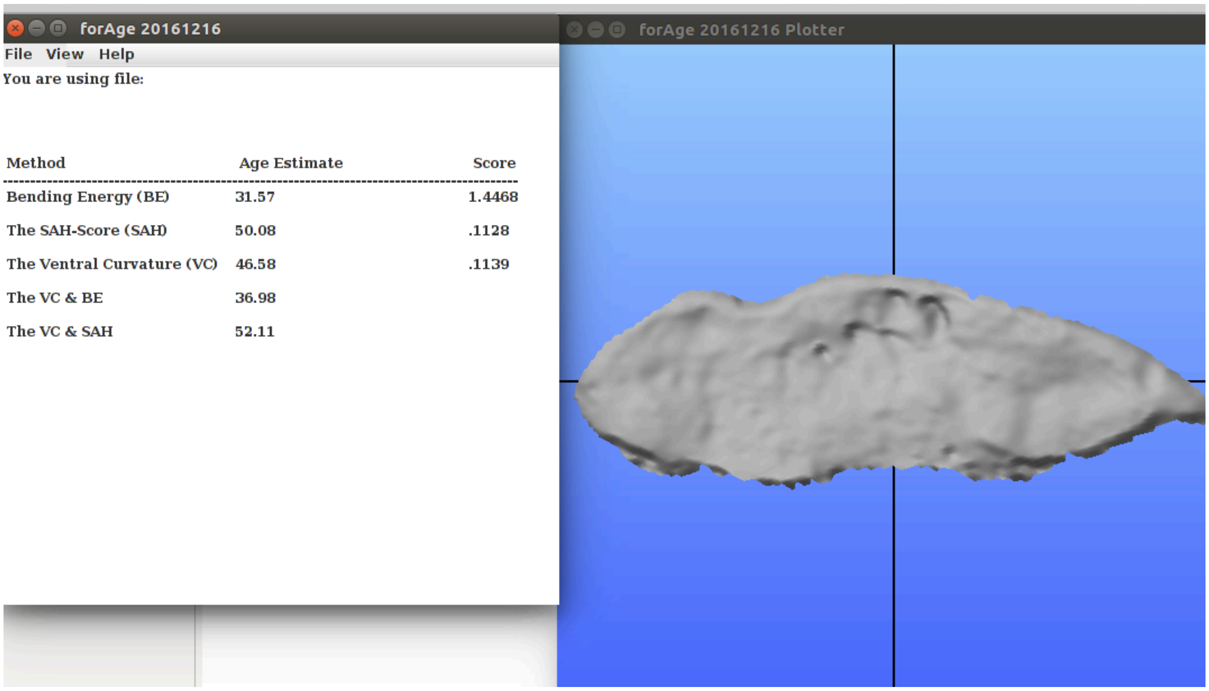

Figure 4-4. The scans were saved as a Ply file, oriented to a standard anatomical position and subjected to the three shape algorithms in the forAge software. Ages and associated scores were produced for each sample.

bending energy required for transforming a perfectly flat thin plate to match the surface of the symphyseal face. The minimum energy that is required for this bending is estimated to account for the changes occurring in the symphyseal phase (i.e., billowed surfaces, flattening, and breakdown of symphyseal phase). The force needed to bend the plate is calculated as the bending energy score, which will be used to estimate age-at-death in the *forAge* software.

The third algorithm follows the protocols of the Curvature of the Ventral Margin (VC) method (Stoyanova et al. 2017), which captures the progressive formation of a rim around the entire symphyseal surface and its later erosion. The algorithm in this technique utilizes least squares to find the best fitting circle through the ventral half of the symphyseal face. The curvature of the ventral margin is measured as 1/r. Thus, the algorithm assumes that a younger individual will have a large radius, and an older individual will display a smaller radius.

After calculating the scores for all three algorithms, the *forAge* software was used to generate individual estimates of age-at-death by regression analysis. Three univariate regression models are based on each raw score- the SAH score, the TPS/BE Score, and the VC Score. In addition, two multivariate regression models are also included in the software. The first multivariate regression model combines the SAH and VC methods, and the second model combines the TPS/BE and the VC methods. As previously mentioned, all five computational models were originally created with a sample of 93 White American males. It is worth mentioning that in contrast to the Suchey-Brooks method, the *forAge* software only provides point estimates of age for each method and does not include an age range. Providing a point estimate in a medico-legal context is not realistic, as it will eliminate most, if not all of the potential missing cases in consideration. This issue will be address in this study by creating age ranges for each computational method.

Data analysis

Intra-observer error test

Approximately 10% of the total sample for each skeletal collection was re-edited by the author using the accompanying software, Scan Studio HD version 1.3.2., in order to test consistency between the editing stage of data collection. As proposed by Slice and Algee-Hewitt (2017) and Stoyanova et al. (2015, 2017), the tridimensional mesh of the pubic symphysis was isolated from the rest of the pubic symphysis and the surrounding areas of the bone (i.e., tubercle, body of the inferior ramus, and pubic body). After each scan was edited, it was uploaded into the *forAge* software to obtain a point estimate of age. In order to evaluate the intra-observer reliability in scan editing, a two-way intraclass correlation coefficient (ICC) (Cicchetti 1994; Schrout and Fleiss 1979) was calculated for both left and right sides. The ICC model generates a coefficient from 0 to 1, with values closer to 1 indicating higher reliability between samples, and values closer to 0 indicating poor reliability (Table 4-2). In order to obtain a coefficient, the package "irr" in R 3.5.1. was used (R Core Team 2013). The "irr" package produces an ICC value, a *p*-value associated with the coefficient, and a lower and upper 95% confidence interval.

Normality test

The distribution of the entire sample was analyzed to test whether the distribution as a whole deviates from a normal distribution. If the distribution of the data deviates from normality, non-

Table 4-2. Intraclass correlation coefficient interpretations by Cicchetti (1994).

ICC	**Reliability**
<0.40	Poor
0.40-0.59	Fair
0.60-0.74	Good
0.75-1.00	Excellent

parametric tests should be used. The Shapiro Wilk's method was used to test the correlation between the data (i.e., chronological ages and estimated ages using all six methods) and the corresponding normal scores. Histograms and Q-Q plots (quantile-quantile plots) were used to visualize the correlation between the ages and the normal distribution. The statistical program R 3.5.1 (https://www.r-project.org) was used to perform this analysis.

Hypothesis 1: Testing for accuracy and reliability of traditional and computational methods

In order to evaluate the performance of each method on all Hispanic samples, mean age estimates obtained from each method (i.e., Suchey-Brooks method, the Slice-Algee-Hewitt score method (SAH), Bending Energy method (BE), the Ventral Curvature method (VC), and two multivariate models using the SAH+VC and BE+VC methods) were compared to documented chronological ages for each individual. This was done by performing Student's t-test with a Bonferonni correction and a Wilcoxon Rank-Sum test as a non-parametric equivalent. Because simultaneous statistical testing is being performed on the same data set (i.e., chronological age against estimated age on six methods), results having *P* values less than 0.05 just by chance can increase with every test (Type I error). For this reason, a Bonferonni correction was performed to adjust the probability of observing at least one significant result due to chance. In this analysis, the significance cut-off value of *P* was determined by

$$P_{criterion} = \alpha / k \qquad (1)$$

with k being the number of statistical comparisons and α the significance level (Field et al. 2012). In this analysis, one traditional age-at-death method and five computational methods are being considered. Consequently, k equals 6 statistical tests at an α of 0.05, therefore the α altered equals 0.008.

The difference between the chronological age and mean estimated age for all methods was calculated in order to analyze the average age distance. The percentage of age estimates that fall within 5, 10, and 15 years from chronological age were also calculated. Spearman Rho correlation coefficients as a non-parametric equivalent to the Pearson correlation were calculated to determine the relationship between chronological age and estimated mean age for all six methods. Lastly, bias and inaccuracies were calculated for each method using documented chronological ages and mean estimated ages on combined and separate Mexican and Puerto Rican samples. In order to calculate bias values, the sum of the difference between the estimated age of each method and the chronological age was calculated and divided by the sample size (Equation 2). The bias value demonstrates the average over- or under- estimation of aging by each method. The inaccuracy was calculated by the absolute value of the sum of the difference between the estimated age of each method and the chronological age, which is then divided by the sample size (Equation 3). The inaccuracy values provide a measure of the error for each method.

$$Bias = \sum(estimated\ age - chronological\ age)/N \qquad (2)$$

$$Inaccuracy = \sum|estimated\ age - chronological\ age|/N \qquad (3)$$

Bias and inaccuracies were calculated for the pooled sample (Mexico and Puerto Rico), as well as separately. Values were also calculated by age groups with an equal number of individuals in each age group, and for the entire dataset.

Hypothesis 2: Revised statistical models for computational methods

Before attempting to analyze the effects of group specific differences in estimated age from all five computational methods between Mexicans and Puerto Ricans, it is necessary to investigate potential differences between the left and right side of the pubic symphysis. Asymmetry in the pubic symphysis refers to significant differences in bone morphology between paired pubic symphysis (Overbury et al. 2009; Kim et al. 2018). If asymmetric changes are unknown, the estimated age of the unknown individual may differ according to which side of the pubic symphysis is used for age estimation, thus inflating age estimation error. Standard practice in the field of anthropology when establishing new age-at-death methods, or when estimating age-at-death, involves the use of the left side of the pubic symphysis (Lottering et al. 2013; Overbury et al. 2009). In other instances, it has been proposed to use the mean age of the two sides to estimate age-at-death (Schmitt 2004). Nevertheless, asymmetry in the pubic bone morphology is a critical component to consider before establishing new methods.

In order to test for asymmetry, a linear mixed model was performed to examine the relationship between estimated age of each method and side. The goal of performing a linear mixed model is to determine whether estimated age on the left and right sides differ, and if so, by how much. A linear mixed model is a type of regression analysis that considers the variation explained by the independent variable, or fixed effect factor, and the variation that is not explained by the independent variable, or random effect factor (Bates et al. 2015; Field et al. 2012; Winter 2013). A fixed effect factor can be considered a set of data that has been collected from all possible treatment conditions, as for example, sex (males and females) or sides (left and right). A random effect factor can be considered a data set that has several possible treatment conditions, but only a random sample has been collected with the goal of generalizing to the broader population (i.e., different geographical groups with different chronological ages) (Field et al. 2012; Winter 2013).

The linear mixed model is appropriate for testing side differences, as it accounts for multiple responses from the same individual. In a general linear model an important assumption that cannot be violated is independence, such that multiple responses from the same subject cannot be used to examine the relationship between variables, as it cannot be regarded as independent from each other. The linear mixed model resolves this issue by adding a random effect for each individual by assuming a different baseline (in this case age ranges) for each individual. For each model, individual differences are modeled by assigning a different random intercept and slope to each sample. After assigning different random intercepts, each model tests if the slopes for each fixed effect are different, and if so, by how much. The following model was used to test for side differences on each method, in which *(1|individualt)* is the random intercept for each individual and the *method* and *side* variables are explanatory factors for age.

$$age = \ method + sides + (1|individual) \qquad (4)$$

The goal in constructing this model is to understand how well all of the fixed effect factors [method and sides] help in accounting for variation in age for each individual in each sample. Results for each model are presented in an analysis of variance table providing F values and p values for each fixed effect factor and for their interactions. It is worth noting that this analysis was performed on each group separately. Coefficient values, *t* values and *p* values were also analyzed in order to examine significant interactions between sides and their respective linear slopes. Each model was also plotted for ease comparison. The statistical program R 3.5.1

was used to perform this analysis and the package “lme4” was used to perform each linear mixed model.

A linear mixed model was also performed to determine whether estimated age between each group differs. The following model was used to test for group differences on each method, in which *(1|individual)* is the random intercept for each individual and the *method* and *group* variables are explanatory factors for age.

$$age = method + groups + (1|individual) \qquad (5)$$

As previously mentioned, the Spanish group was included in this analysis. Similar to the asymmetry models, an analysis of variance table providing F values and *p* values for each fixed effect factor and for their interactions was included for each analysis. Also, coefficient values, *t* values and *p* values were also analyzed in order to examine significant interactions between groups and their respective linear slopes. Each model was also plotted for simplicity when comparing all three groups.

Finally, after examining side and group differences, new regression equation models were created dependent on the group differences observed on the linear mixed models previously discussed. A linear regression analysis with an inverse calibration (x on y) was performed to quantify relationship between the estimated age for each method and their respective scores provided by the *forAge* software. Previous studies have demonstrated that the regression of an age-indicator on age provides unbiased estimates when using an estimate from a population with a similar distribution (Konigsberg and Frankenberg 1997; Konigsberg et al 1998). Thus, for this analysis, age was regressed on the indicator, with age being the dependent variable and score provided by the *forAge* software the independent variable.

A second set of regression equations were performed using a log transformation on the raw score to make the age variable more homoscedastic. Several studies examining the utility of computational methods have shown that the score variable regressed on age presents issues with homoscedasticity and a log transformation usually helps resolve this issue (Slice and Algee-Hewitt 2015; Stoyanova et al. 2015,2017). For each equation, the R^2 and Root Mean Square Error (RMSE) were reported in order to examine how well each model performs. The R^2 provides a measure of how much variance is explained by the model and the RMSE provides information regarding how accurate the model predicts the response variable (age). The RMSE value is in the same unit as the response variable (age=years), thus it can be used to quantify the amount of error for the model. Lower RMSE values and higher R^2 values indicate a better fit to the model.

Several regression equations were created, including equations for (1) the pooled sample from Mexico, Puerto Rico, and Spain and (2) the two samples with the largest sample sizes: Puerto Rico and Spain. By creating a regression equation with two groups, the third sample, in this case the sample with the smallest *n*, was used for cross validation analysis. Bias and inaccuracies were calculated to test the accuracy and reliability of the new regression equations on the sample with the smallest sample size.

The next two chapters will present the results obtained for each aging techniques (traditional and computational) on the Mexican and Puerto Rican samples. In addition, results from the linear mixed model analysis for asymmetry and group differences will be presented. Finally, newly developed computational equations for the estimation of age are established. Their respective associated error rates and validation analysis are also presented.

CHAPTER FIVE
RESULTS: HYPOTHESIS ONE

This chapter provides an analysis of the data used to evaluate hypothesis one, which was previously proposed in the introductory chapter. Hypothesis one states that various methods, originally developed on non-Hispanic samples, will yield age estimates with higher error rates for Hispanic samples than when using samples of European background. The methods include the Suchey-Brooks method, the Slice-Algee-Hewitt Score method (SAH), the Thin-Plate Splines/Bending Energy method (TPS/BE), the Ventral Curvature method (VC), and two multivariate methods (TPS/BE+ VC and SAH Score + VC) of the pubic symphysis. In order to analyze this hypothesis, several statistical analyses were performed to test for differences between the chronological and estimated age of each sample. Bias and inaccuracy levels between chronological and mean estimated ages and the percent of age estimates that fall within 5, 10, and 15 years of the chronological age were calculated. Finally, the correlation between chronological ages and estimated ages on all samples were analyzed. The aforementioned analyses were conducted on a pooled sample with Mexican and Puerto Ricans combined, as well as separately for each sample.

Intra-observer error test

Before data analysis, observer consistency in data manipulation prior to obtaining an estimated age was evaluated using a two-way random intraclass correlation coefficient (ICC) (Cicchetti 1994; Cicchetti and Sparrow 1981; Schrout and Fleiss 1979) available in the 'irr" package for R 3.5.1 statistical software. In order to obtain an age estimate from the Slice-Algee-Hewitt Score method (SAH), the Thin-Plate Splines/Bending Energy Method (TPS/BE), and the Ventral

Curvature method (VC) of the pubic symphysis, the tridimensional mesh of the symphyseal surface needs be isolated from the rest of the pubic bone and the surrounding areas of the bone (i.e., tubercle, body of the inferior ramus, pubic body) by manual manipulation with the NextEngine software. Thus, it is important to examine the consistency of the observer in isolating the pubic symphysis prior to obtaining an age estimate. In this project, the ICC model was used to evaluate the consistency between multiple scans on the same sample by the same observer. The ICC model generates a coefficient from 0 to 1, with values closer to 1 indicating a higher reliability. The guidelines suggested by Cicchetti and Sparrow (1981) and Cicchetti (1994) were used to interpret the ICC coefficient in relation to reliability.

Table 5-1 provides the results of the ICC for both right and left sides separately. Results show that nearly all of the aging methods were in excellent agreement. The VC+SAH model presented an ICC of .799-.747 for the right and left side, respectively, which is considered borderline excellent. These results are consistent with Kim and colleagues (2018), who demonstrated that methods that examine the ventral curvature of the pubic symphysis tend to be more sensitive to observer error than the SAH and the BE methods that capture the complexity of the symphyseal face topography.

Normality test

Prior to data analysis, the age distribution of the entire sample was analyzed to test whether the distribution as a whole deviates from a normal distribution. The Shapiro-Wilk's test was used to compare the chronological and estimated age of all samples to a normally distributed set of scores with the same mean and standard deviation. When analyzing the chronological age for the Puerto Rican and Mexican samples together, a significant value ($p<0.05$) is obtained, thus indicating a deviation from normality. The ages estimated with the Ventral Curvature method (VC) and the Suchey-Brooks method (SB) also presented a significant value, therefore deviating from a normal distribution. (Table 5-2). A histogram and Q-Q plots (Figure 5-1) of the data confirm the Shapiro-Wilk's test results for the pooled sample. As presented in the histogram, the distribution of the chronological age for the pooled sample is not symmetrical with most of the sample skewed towards younger individuals. The Q-Q plot for the chronological age presents a curved line throughout, representative of a non-normal distribution.

Table 5-1. Intra-observer error: ICC absolute agreement of within-observer age estimates derived from first and second editing.

Estimate for each method	**Right**				**Left**			
	ICC	p-Value	Lower 95%	Upper 95%	ICC	p-Value	Lower 95%	Upper 95%
SAH Score (SAH)	.932	3.15e-12	0.991	0.999	.988	2.05e-19	0.994	0.999
TPS/Bending Energy (TPS/BE)	.975	5.64e-14	0.947	0.989	.988	7.51e-14	0.966	0.996
Ventral Curvature (VC)	.883	2.86e-04	0.735	0.922	.945	6.68e-09	0.848	0.981
VC + SAH	.799	0.00002	0.542	0.925	.747	0.000308	0.411	0.907
VC + TPS/BE	.944	6.84e-9	0.976	0.999	.969	8.33e-11	0.914	0.999

Table 5-2. Results for the Shapiro Wilk's test for the pooled sample (* =deviation from normality).

Method	**W**	***p*-value**
Chronological Age	0.894	2.863e-09*
Estimated age with SAH Score (SAH)	0.987	0.187
Estimated age with TPS/Bending Energy (TPS/BE)	0.988	0.213
Estimated age with Ventral Curvature (VC)	0.972	0.003*
Estimated age with VC+BE	0.981	0.033*
Estimated age with VC + SAH	0.995	0.921
Estimated age with Suchey-Brooks	0.909	2.251e-08*

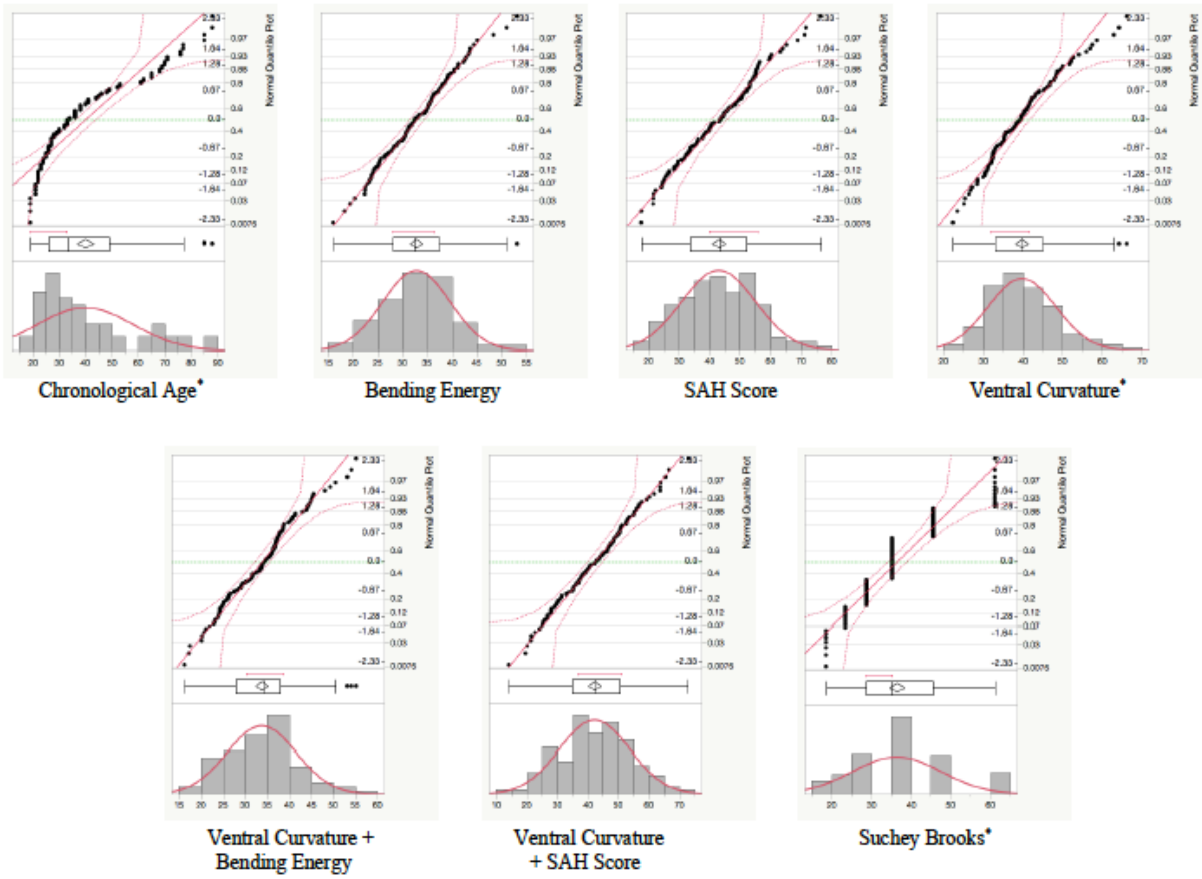

Figure 5-1. Histogram and Q-Q plot for the pooled sample (Puerto Rico and Mexico) on the chronological age and estimated age-at death with all six methods (* =deviation from normality)

As one of the main goals of this study involves comparing age-at-death estimates between groups, it is important to examine the age distribution in each group separately. When the sample was analyzed by groups, the chronological age-at-death for both Mexican and Puerto Rican samples presented a significant value ($p<0.05$), indicating a deviation from normality. The age estimated with the Suchey-Brooks method was also significant for both groups. For the Puerto Rican sample, the estimated age using the VC method was also significant, and therefore, deviated from normality (Table 5-3). A histogram and Q-Q plots of the chronological age for the Puerto Rican and Mexican samples is presented separately in Figures 5-2 and 5-3. The histogram presenting the distribution of the chronological age of the Puerto Rican sample is not symmetrical, and it is skewed towards younger individuals (i.e., 20-40 years of age). The histogram for the chronological age of the Mexican sample presents a slightly different pattern with the sample distribution not equally symmetrical. As some of the data for the Puerto Rican and Mexican samples are not normally distributed, non-parametric procedures were used in the analyses.

Table 5-3. Results for the Shapiro Wilk's test for each sample (* =deviation from normality)

Method	**Puerto Rico**		**Mexico**	
	W	*p*-value	W	*p*-value
Chronological Age	0.868	6.46e-09*	0.936	0.026*
Estimated age with SAH Score (SAH)	0.988	0.433	0.963	0.222
Estimated age with TPS/Bending Energy (TPS/BE)	0.993	0.846	0.969	0.334
Estimated age with Ventral Curvature (VC)	0.971	0.011*	0.972	0.425
Estimated age with VC+BE	0.984	0.179	0.991	0.991
Estimated age with VC + SAH	0.994	0.937	0.970	0.493
Estimated age with Suchey-Brooks	0.887	4.57e-08*	0.807	8.91e-05*

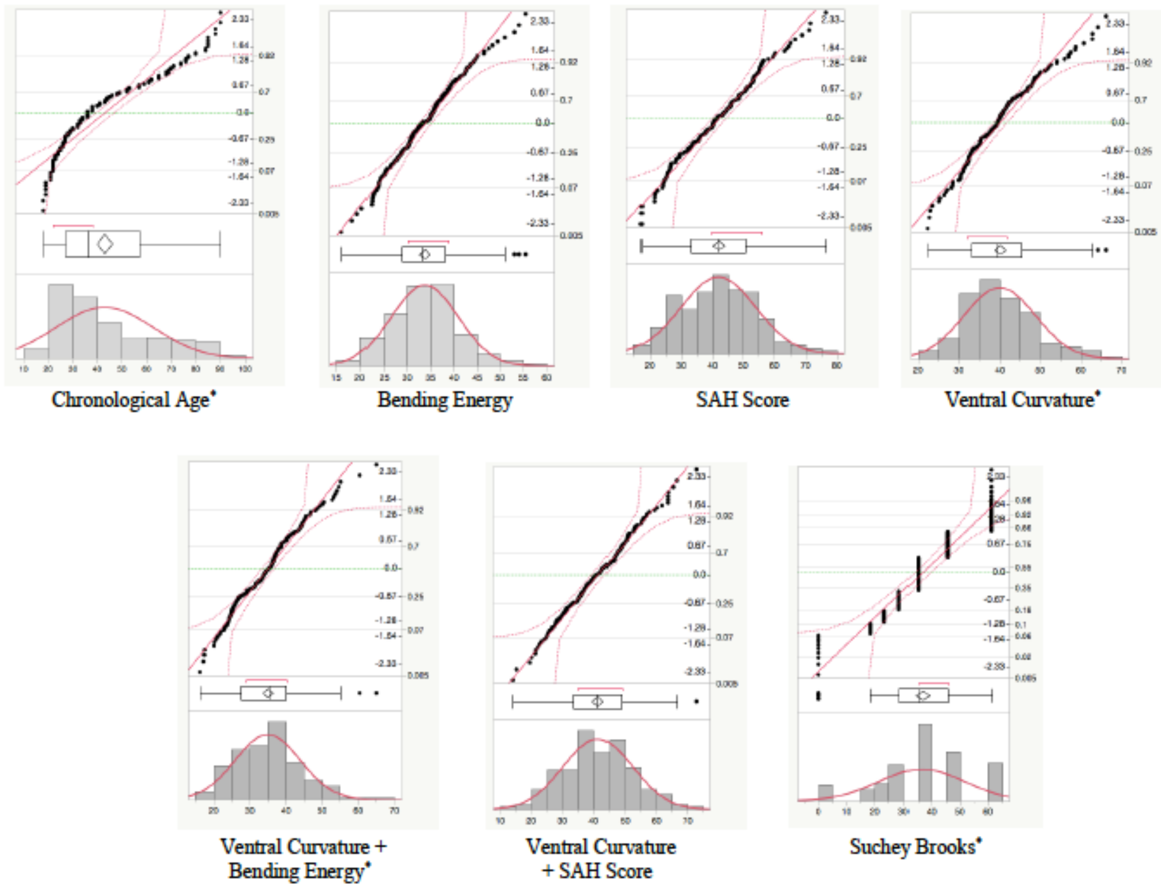

Figure 5-2. Histogram and Q-Q plot for the Puerto Rican sample on the chronological age and estimated age-at death with all six methods (* =deviation from normality).

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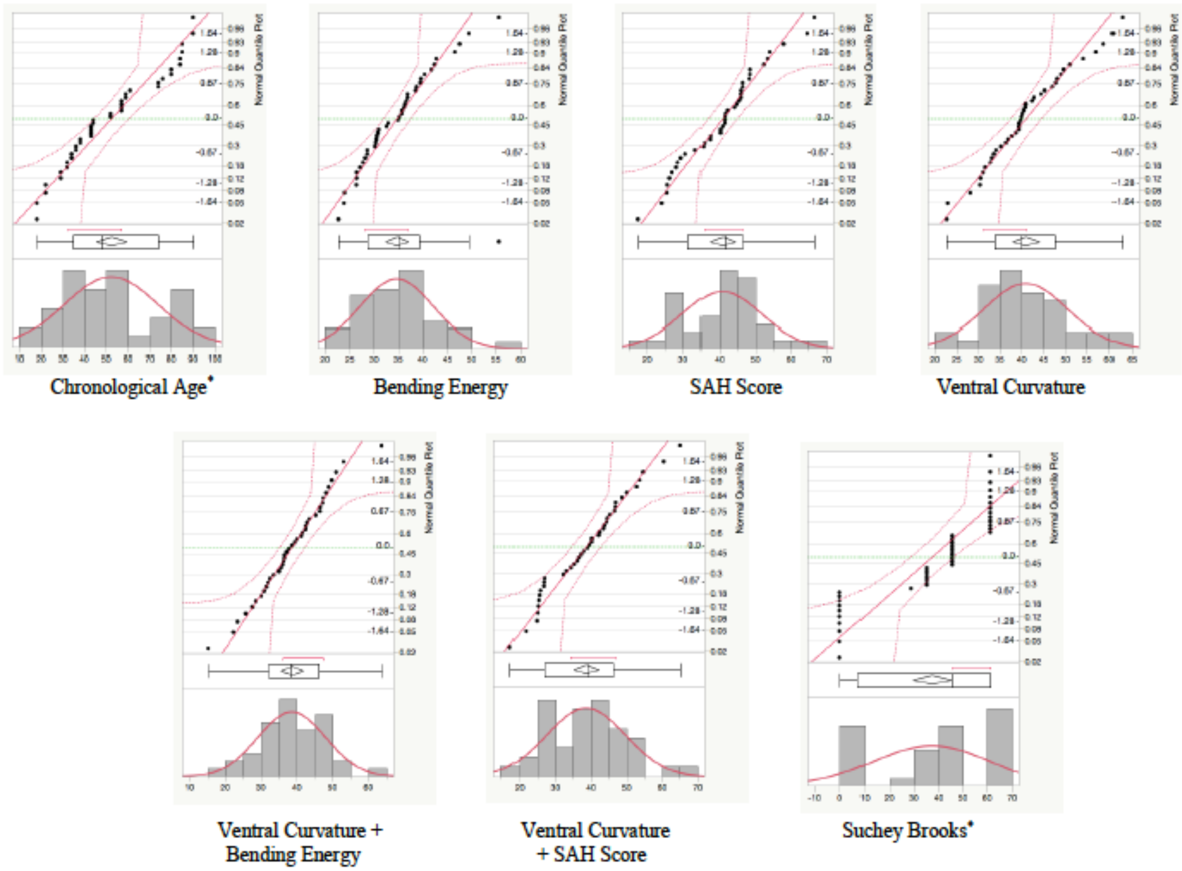

Figure 5-3. Histogram and Q-Q plot for the Mexican sample on the chronological age and estimated age-at death with all six methods (* =deviation from normality).

Testing for accuracy and reliability: Bias and inaccuracy

Hypothesis one states that methods originally developed on non-Latin American samples, will yield age estimates with higher error rates for samples originating from Latin America. The methods include the Suchey-Brooks method, the Slice-Algee-Hewitt Score method (SAH), the Thin-Plate Splines/Bending Energy method (TPS/BE), the Ventral Curvature method (VC), and two multivariate methods (TPS/BE+ VC and SAH Score + VC) of the pubic symphysis. This examination will quantify the magnitude of error between all techniques and determine the appropriateness of each method on groups of Puerto Rican and Mexican background.

Pooled sample (Puerto Rico and Mexico)

To determine whether the estimated mean ages calculated from all six methods were significantly different from the chronological ages, a Student's t-test with a Bonferonni correction (α=0.05, $\alpha_{altered}$=0.008) and a Wilcoxon Rank-Sum Test as a non-parametric equivalent were performed. Results indicate that chronological age and estimated age did not differ significantly using the SAH method (*p*-value =.144), the VC method (*p*-value=.429), the TPS/BE method + VC method (*p*-value=.042), the SAH + VC method (*p*-value =.187), and the Suchey-Brooks method (*p*-value =.323). The TPS/BE method (*p*-value=3.42e-06) was the only method that presented significant differences between chronological age and estimated age. Table 5-4 presents the results of the Student's t-test for paired samples with a Bonferonni correction (α=0.05, $\alpha_{altered}$=0.005), and a Wilcoxon Rank-Sum Test for the pooled sample.

The estimated difference between the chronological age and the estimated age for each individual was calculated for all aging methods. The mean age difference for the SAH score, the VC method, and the SAH + VC method respectively are -3.12, -2.95, and -3.13 years for the

Table 5-4. Student's t-test for paired samples with a Bonferroni correction, and a Wilcoxon Rank-Sum Test-as a non-parametric equivalent for the pooled sample (* =significantly different with a α=0.05 (α = 0.008))

Method	***p*-value**	**W or t value**
SAH Score (SAH)	.144	1.472
TPS/Bending Energy (TPS/BE)	3.421e-06*	4.992
Ventral Curvature (VC)	.429	3044
TPS/BE+VC	.042	3888
SAH+VC	.187	1.328
Suchey-Brooks	.323	3575

pooled sample. The corresponding numbers for the Suchey-Brooks method, the TPS/BE+VC method, and the TPS/BE method respectively are -6.27, -8.67, and -10.27 years. When comparing the average age distance between all aging methods, the BE method tends to have the greatest mean distance. All age distances are represented by negative values, thus indicating that all aging methods tend to underestimate age for the pooled sample.

In addition, results indicate that 25-45% of the age estimates are within five years of the chronological age for the pooled sample on all six methods, while 19-26% of the age estimates fall within 6 to 10 years of the chronological age. Overall, more than half of the estimates for all aging methods are within 15 years or less of the chronological age. Table 5-5 presents the aforementioned results in more detail.

Spearman Rho correlations were calculated to assess the strength of the relationship between chronological age and estimated age on all aging methods. Results indicate that chronological age and the estimated age for the Suchey-Brooks method is the highest at .72, followed by the TPS/BE+VC method (.48), the SAH+VC method (.44), the SAH Score method (.41), the TPS/BE method (.40), and the VC method (.31). All correlations differed significantly from 0 at a $\alpha = 0.05$. Spearman Rho Rank correlations results are included in Table 5-6.

Inaccuracy and bias were calculated for each aging method as a way to assess the accuracy of each method. When the sample was pooled by group, the SAH method and the VC+SAH Score method presented the smallest bias (-2.53 years, -2.71 years, respectively), while the TPS/BE method had the greatest bias (-10.86 years). All six methods tended to underestimate age when both Mexican and Puerto Rican samples were analyzed together. The Suchey-Brooks method, the VC +TPS/BE method, and the TPS/BE method underestimated age

Table 5-5. For each method, the estimated percent of age that falls within 5, 10, and 15 years of the chronological age for the pooled sample.

PR + Mexico						
Difference in years	**TPS/Bending Energy (TPS/BE)**	**SAH Score**	**Ventral Curvature (VC)**	**TPS/BE+ VC**	**SAH+ VC**	**Suchey-Brooks**
≤5	32.09%	29.62%	24.69%	32.09%	28.39%	44.44%
6 to 10	22.22	20.98	18.51	25.92	22.22	19.75
11 to 15	9.87	16.04	17.28	11.11	14.81	7.40
≥16	38.27	33.33	39.50	30.86	33.33	28.39

Table 5-6. Mean values of the age-estimates for each computational method and the Spearman's rank correlation between the chronological age and the estimated age for the pooled sample (* =significantly different with a α=0.05)

Method	**Mean**	**Correlation**	**Sign. Prob.**
Chronological Age	43.28	-	-
TPS/Bending Energy	33.01	0.404	.0001*
SAH Score	40.16	0.410	.0001*
Ventral Curvature	40.33	0.316	.004*
TPS/Bending Energy + Ventral Curvature	34.61	0.485	4.41e-06*
SAH + Ventral Curvature	40.15	0.441	3.724e-05*
Suchey-Brooks	37.01	0.722	2.7e-14*

with the greatest bias (-4.60 years, -9.08 years, and -10.86 years, respectively). Results are presented in descending order in Table 5-7. When the bias results were plotted for all six methods separately, it can be noted that the age of individuals under 40 years tends to be overestimated, while trends for underestimation began for individuals over 40 years of age, with bias increasing as age increases.

In contrast to the bias results, the Suchey-Brooks method had the smallest inaccuracy levels, followed by the SAH score and the VC+SAH score method. The TPS/BE method and VC method had the largest inaccuracy levels. When the sample was divided into age groups (Table 5-8), bias and inaccuracies increased with age. As seen in the scatter plots (Figure 5-4 to 5-9), the majority of the methods tend to underestimate age for individuals between 35 years and above. Individuals at the age group of 35-45 presented the least bias and inaccuracy, with results ranging between -0.06 years to -6.27 years for bias levels and 3.46 years to 4.34 years for inaccuracy levels.

Table 5-7. Bias and inaccuracy values calculated for the pooled sample.

Method	**Bias**	**Inaccuracy**
SAH Score	-2.53	13.87
SAH+ VC	-2.71	13.66
Ventral Curvature	-2.95	15.47
Suchey-Brooks	-4.60	9.98
TPS/BE+ VC	-9.08	14.46
TPS/Bending Energy	-10.86	15.58

Table 5-8. Bias and inaccuracy values calculated for each method, by age group, on the pooled sample.

	SAH Score		**TPS/BE**		**Ventral Curvature**		**SAH + Vent Curv**		**TPS/BE + Vent Curv**		**Suchey Brooks**	
Age At death	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.
<25	2.69	3.51	1.81	3.03	6.67	6.67	1.49	2.37	2.69	3.33	1.71	4.14
26-34	6.47	6.80	2.08	3.39	5.08	5.16	2.41	3.62	6.37	6.62	6.57	7.06
35-45	0.28	3.75	-3.61	3.82	1.01	4.20	-2.66	4.34	0.80	3.46	-0.18	3.97
46-65	-3.82	5.22	-9.9	9.9	-6.66	6.89	-7.94	8.25	-4.23	4.78	-9.75	10.46
≥65	-23.39	23.39	-17.19	17.19	-18.79	18.79	-21.37	21.37	-17.84	17.84	-26.25	26.25

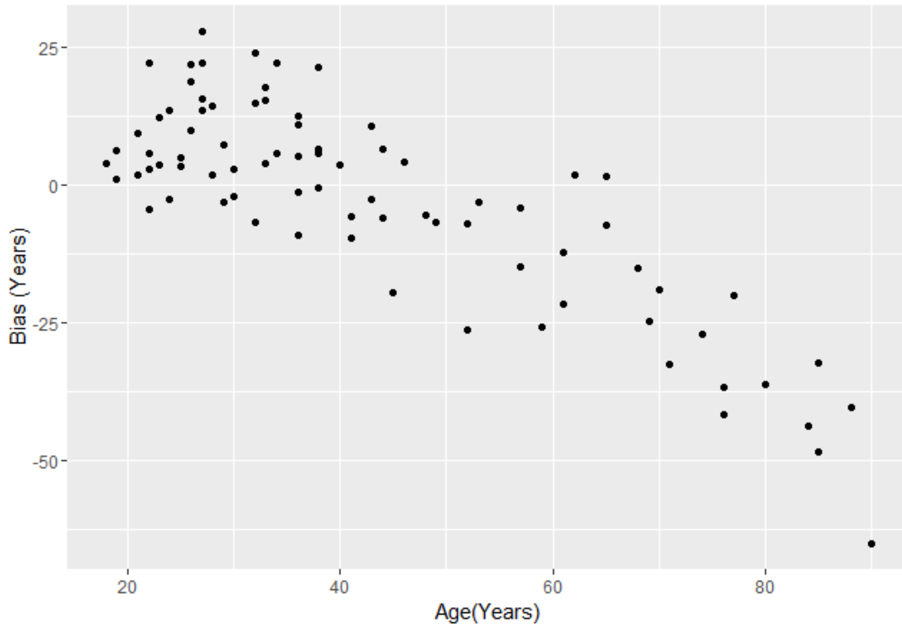

Figure 5-4. Bias for the Slice-Algee-Hewitt Score method for the pooled sample.

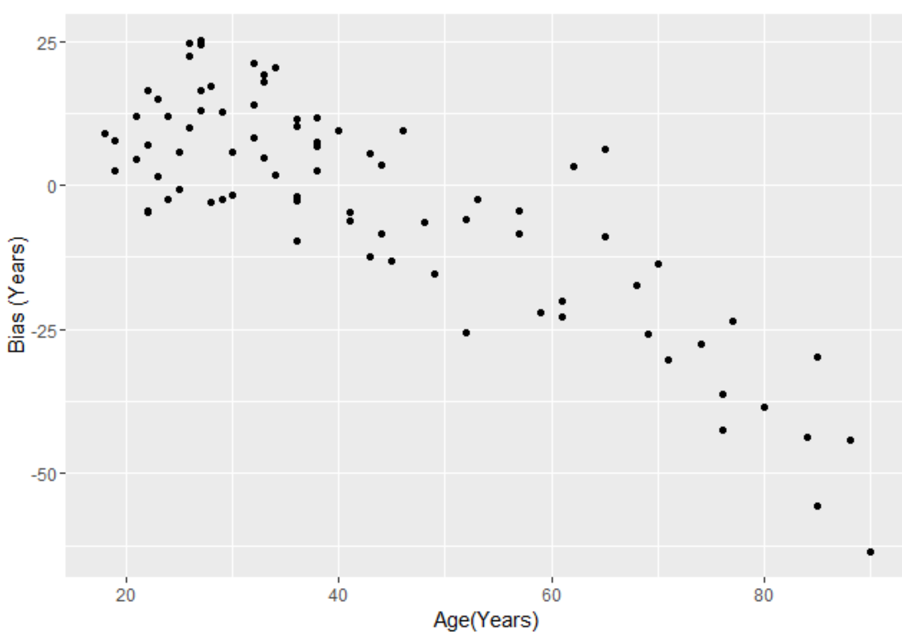

Figure 5-5. Bias for the Ventral Curvature + Slice-Algee-Hewitt Score method for the pooled sample.

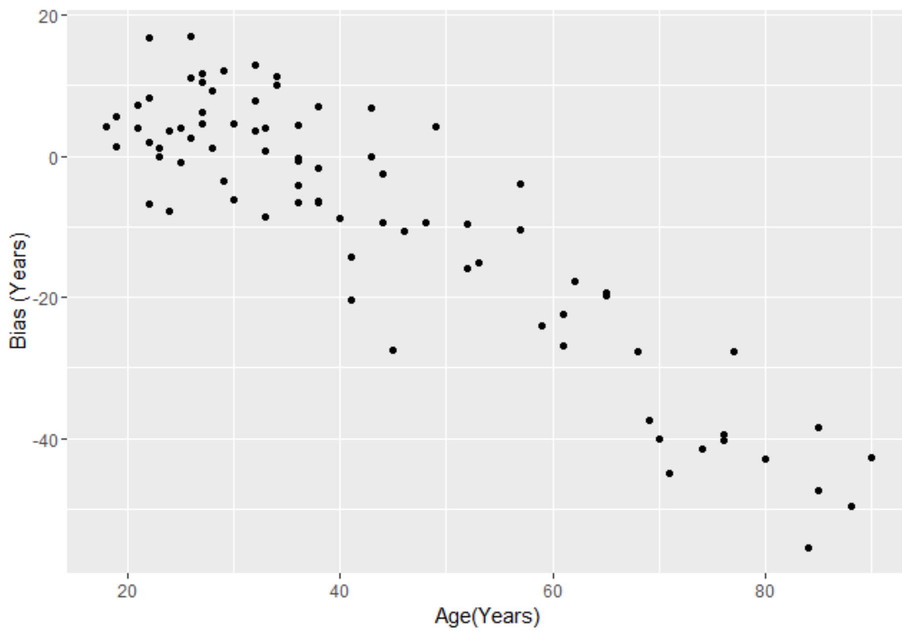

Figure 5-6. Bias for the Ventral Curvature method for the pooled sample.

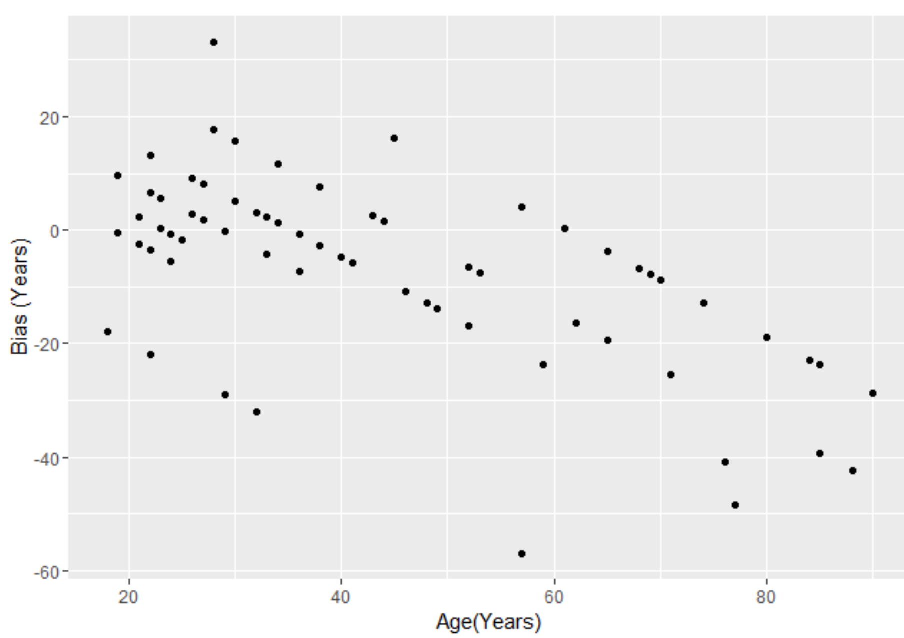

Figure 5-7. Bias for the Suchey-Brooks method for the pooled sample.

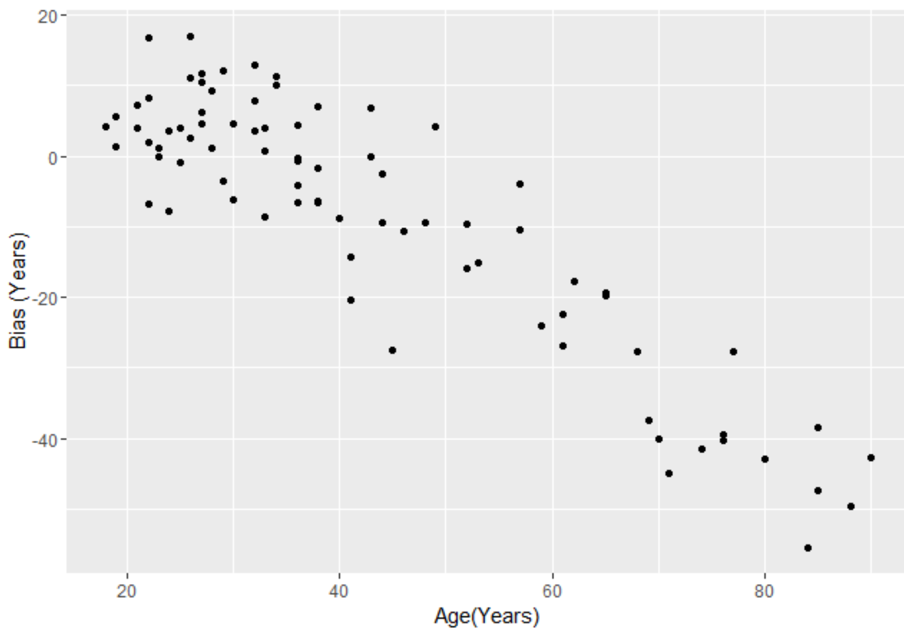

Figure 5-8. Bias for the Ventral Curvature + Bending Energy method for the pooled sample.

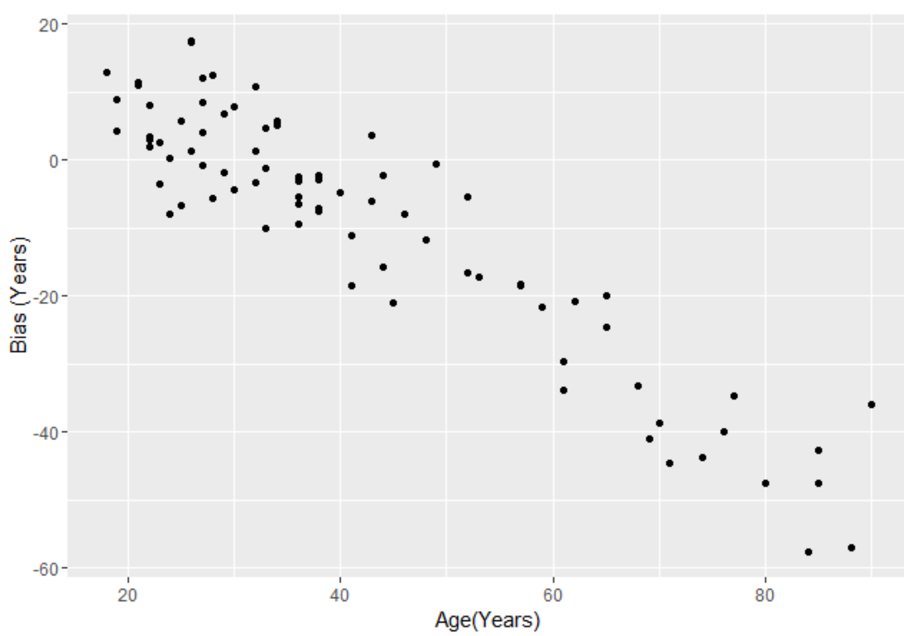

Figure 5-9. Bias for the TPS/Bending Energy method for the pooled sample.

Puerto Rican sample

For the Puerto Rican sample, no significant differences exist between chronological age and estimated age when using the SAH score (*p*-value=.463), the VC method (*p*-value=.038), the VC + SAH method (*p*-value=.468), and the Suchey-Brooks method (*p*-value=.839). For the TPS/BE method (*p*-value=.000) and the VC + TPS/BE method (*p*-value=.003) significant differences were observed between chronological age and estimated age. Results are presented in Table 5-9, including results for both sides pooled, and both right and left side separately.

The average age distance between the chronological age and the estimated age for the VC method, the VC + SAH method, and the SAH method respectively are 0.40, 1.46, and 1.49 years. The corresponding numbers for the Suchey-Brooks method, the VC + TPS/BE method, and the TPS/BE method are -3.36, -6.57, and -7.99 years, respectively. Similar to the average age distance of the pooled sample, the TPS/BE method remains the method that produces the largest distance between chronological age and estimated age. Furthermore, results indicate that 12-50% of the age estimates are within 5 years of the chronological age when both sides are pooled on all six models, while 20-28% of the age estimates fall within 6 to 10 years of the chronological age. As previously seen when both groups were pooled, more than half of the estimates for all aging methods are within 15 years or less of the chronological age. Table 5-10 presents the results in more detail.

Spearman Rho correlations improved for most of the methods in comparison to the pooled sample (Mexicans and Puerto Ricans). Results indicate that chronological age and estimated age for the Suchey-Brooks method, the VC+SAH method, and the SAH score are the highest at .73, .56, and .57, respectively. In comparison to the pooled results, the SAH score

Table 5-9. Student's t-test for paired samples and a Wilcoxon Rank-Sum Test-as a non-parametric equivalent for the Puerto Rican sample (* =significantly different with a α=0.05 (αaltered=0.008))

Method	***p*-value**	**W or t value**
SAH Score	.463	-.738
SAH+ VC	.000*	3.620
Ventral Curvature	.038	1405
Suchey-Brooks	.003*	3.054
TPS/BE+ VC	.468	0.729
TPS/Bending Energy	.839	1839

Table 5-10. For each method, the percent of age estimated that falls within 5, 10, and 15 years of the chronological age for the Puerto Rican sample.

Puerto Rico						
Difference in years	**TPS/Bending Energy (TPS/BE)**	**SAH Score**	**Ventral Curvature (VC)**	**TPS/BE+ VC**	**SAH+ VC**	**Suchey-Brooks (SB)**
≤5	33.03%	11.61%	22.32%	26.79%	14.29%	50.0%
6 to 10	27.67	19.64	23.21	24.11	22.32	25.0
11 to 15	13.39	18.75	16.07	11.61	15.18	8.93
>15	25.89	50.00	41.07	37.50	48.21	16.1

correlations increased the most, ranging from .40 when the sample was pooled to .57 when the Mexican sample was removed, and the Puerto Rican sample was examined alone. These results are followed by the VC + TPS/BE (.45), the TPS/BE method (.38), and the VC method (.28). Similar to previous results, all correlations were significant at a α =0.05 (Table 5-11).

When the Puerto Rican sample was analyzed on its own, the Suchey-Brooks method, TPS/BE, and VC+BE methods tended to overestimate age the most. The method that presented the least amount of bias was the VC method with 0.40 years. The Suchey-Brooks method, the VC + TPS/BE method, and the TPS/BE method presented the greatest amount of bias. The method with the greatest bias was the TPS/BE method, which tended to underestimate age. Table 5-12 presents the bias and inaccuracy results in more detail.

As previously seen when inaccuracies were calculated on the pooled sample, the SB method presented the least amount of inaccuracies. Similar inaccuracy results were seen for the SAH score, the SAH + VC method, the TPS/BE method, and the TPS/BE + VC method. The largest inaccuracy was documented on the VC method (15.39 years). Inaccuracies and bias increased with age when the sample was examined by age groups. The least amount of bias and inaccuracies for all six methods was observed in groups of individuals 35-45 years of age. Bias and inaccuracies increase significantly in individuals ages 65 and above. Overall, results show that bias and inaccuracies in all six methods increased in individuals 30 years of age or younger, and individuals 50 years of age and older. Table 5-13 presents the bias and inaccuracy values by age group in more detail.

Table 5-11. Mean values of the age-estimates for each computational method and the Spearman's rank correlation between the chronological and estimated age for the Puerto Rican sample (* =significantly different with an α=0.05)

Age-at-Death	**Mean**	**Correlation**	**Sign. Prob.**
Chronological Age	39.87	-	-
TPS/Bending Energy	31.87	0.385	0.002*
SAH Score	41.36	0.563	2.781e-06*
Ventral Curvature	40.27	0.284	0.0293*
TPS/Bending Energy + Ventral Curvature	33.29	0.454	0.0002*
SAH +Ventral Curvature	41.33	0.573	1.65e-06*
Suchey-Brooks	36.51	0.732	2.99e-11*

Table 5-12. Bias and inaccuracy values calculated for each method on the Puerto Rican sample.

Method	**Bias**	**Inaccuracy**
Suchey-Brooks	-3.36	9.16
SAH Score	1.49	12.34
TPS/Bending Energy	-7.99	13.36
SAH Score +Ventral Curvature	1.46	12.04
TPS/Bending Energy + Ventral Curvature	-6.57	12.70
Ventral Curvature	0.40	14.91

Table 5-13. Bias and inaccuracy values calculated for each method, by age group, on the Puerto Rican sample.

	SAH Score		**TPS/BE**		**Ventral Curvature**		**SAH + Vent Curv**		**TPS/BE + Vent Curv**		**Suchey Brooks**	
Age At death	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.
<25	2.69	3.51	1.81	3.03	6.67	6.67	1.49	2.37	1.49	2.37	0.85	2.07
26-34	6.47	6.80	2.08	3.39	5.08	5.16	2.41	3.62	6.37	6.62	3.15	3.40
35-45	0.28	3.75	-3.61	3.82	1.01	4.20	-2.66	4.34	0.80	3.46	-0.69	2.17
46-65	-3.82	5.22	-9.9	9.9	-6.66	6.89	-7.94	8.25	-4.23	4.78	-5.61	5.63
≥65	-17.19	17.19	-23.39	23.39	-18.79	18.79	-21.37	21.37	-17.84	17.84	-13.71	13.71

Mexican sample

When the Mexican sample was analyzed, all computational methods showed significant differences between chronological and estimated age. The Suchey-Brooks method was the only method that did not differ significantly between chronological age and estimated age.

When the difference between chronological age and estimated age was calculated for the Mexican sample, higher values were encountered than previously presented on the pooled sample and the Puerto Rican sample alone. The mean age distance ranged from -12.55 years when using the VC method, followed by the Suchey-Brooks method (-14.59 years), the VC+TPS/BE method (-14.67 years), the VC+SAH method (-16.27 years), and the SAH score (-16.32 years). Once again, the TPS/BE method presented the highest average age distance of -16.75 years between chronological age and estimated age. Overall, all methods tend to underestimate age for the Mexican sample. In addition, results indicate that 23-37% of the age estimates are within 5 years of the chronological age. In contrast to the Puerto Rican sample, more than half of the estimates are within 10 years of the chronological age. Spearman Rho correlations were non-significant for all five computational methods. The Suchey-Brooks method presented a correlation value similar to the previous results, .73, and significant at α of 0.05. Tables 5-14 to 5-16 present the aforementioned results in more detail.

In contrast to the Puerto Rican sample, the Mexican sample presents higher bias and inaccuracy values. However, trends regarding the performance of each method are similar to previous results for the Puerto Rican sample. The Suchey-Brooks method has the least amount of bias, with -9.27 years. As in other analyses, the BE method remains the one with the greatest bias

Table 5-14. Student's t-test for paired samples, and a Wilcoxon Rank-Sum Test- as a non-parametric equivalent for the Mexican sample (* =significantly different with a (α altered=0.008))

Method	***p*-value**	**W or t value**
SAH Score (SAH)	.000*	3.736
TPS/Bending Energy (TPS/BE)	3.107e-05*	4.710
Ventral Curvature (VC)	.002*	3.172
VC + TPS/BE	.000*	3.976
VC + SAH	.000*	3.921
Suchey-Brooks	.312	261

Table 5-15. For each method, the percent of age estimated that falls within 5, 10, and 15 years of the chronological age for the Mexican sample.

Mexico						
Difference in years	**TPS/Bending Energy (TPS/BE)**	**SAH Score**	**Ventral Curvature (VC)**	**TPS/BE+ VC**	**SAH+ VC**	**Suchey-Brooks (SB)**
≤5	32%	26%	37%	29%	23%	37.5%
6 to 10	21	21	21	32	23	12.5
11 to 15	0	16	5	13	26	12.5
>15	47	37	37	26	29	43.7

Table 5-16. Mean values for the age estimates for all six methods and the Spearman Rho correlation between the chronological age and the estimated age for the Mexican sample (* =significantly different with a $\alpha=0.05$).

Age-at-Death	**Mean**	**Correlation**	**Sign. Prob.**
Chronological Age	53.05		
TPS/Bending Energy	36.30	0.234	0.306
SAH Score	36.73	0.129	0.576
Ventral Curvature	40.50	0.367	0.101
Ventral Curvature + TPS/Bending Energy	38.38	0.287	0.205
Ventral Curvature + SAH	36.78	0.293	0.196
Suchey-Brooks	38.46	0.739	0.000*

(-16.74 years). It is worth noting that all methods tend to underestimate age on the Mexican sample.

Inaccuracy values present a similar pattern as do the bias results, with the Suchey-Brooks method presenting the least amount of inaccuracy and the TPS/BE method presenting the highest value. In contrast to previous inaccuracy results, these are the highest values presented so far, with values as high as 20.57 years. When the sample was divided into age groups, individuals between 34 to 50 years of age presented the smallest inaccuracy values on all six methods, ranging from 3.02 years to 8.70 years. Similar to the pooled sample and the Puerto Rican sample, a trend of increasing inaccuracy with age was observed. Individuals over 90 years of age presented the largest inaccuracy values (23.55 years to 50.34 years). Tables 5-17 and 5-18 present these results in greater detail.

Table 5-17. Bias and inaccuracy values calculated for each method on the Mexican sample.

Method	**Bias**	**Inaccuracy**
Suchey-Brooks	-9.27	13.07
Ventral Curvature	-12.54	17.09
SAH Score	-16.32	20.57
SAH Score + Ventral Curvature	-16.27	19.47
TPS/Bending Energy +Ventral Curvature	-14.66	18.29
TPS/Bending Energy	-16.74	19.64

Table 5-18. Bias and inaccuracy values calculated for each method, by age group, on the Mexican sample.

	SAH Score		**TPS/BE**		**Ventral Curvature**		**SAH + Vent Curv**		**TPS/BE + Vent Curv**		**Suchey Brooks**	
Age At death	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.	Bias	Inacc.
<33	5.42	7.25	4.69	6.06	5.66	6.28	1.01	5.58	4.88	7.61	11.6	11.6
34-50	-3.50	8.70	-2.03	3.46	2.78	4.41	-0.17	7.03	-0.30	3.02	2.72	3.04
50-76	-21.55	21.55	-25.91	25.91	-19.11	19.11	-21.75	21.75	-21.63	21.63	-13.23	14.7
≥90	-50.34	50.34	-45.9	45.9	-42.90	42.90	-48.4	48.4	-44.86	44.86	-23.55	23.55

CHAPTER SIX
RESULTS: HYPTHESIS TWO

Hypothesis two is based on findings of Gonzalez Buchard and colleagues (2005) and several other studies suggesting that Hispanic groups could be sufficiently different (e.g., genetically and morphologically, to the extent of being distinguishable between groups (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Hughes et al. 2018; Moreno-Estrada et al. 2013,2014; Via et al. 2011). Thus, the second hypothesis proposes that age estimation models developed for Hispanic samples will perform better if they are population specific. This hypothesis will address whether or not Mexican and Puerto Rican samples should be separated as two different populations, and in turn, require two different age-at-death techniques, or if they should be pooled together in order to develop a single age-at-death technique. This hypothesis will further address questions related to the effects of group specific and individual life history differences in senescence and its consequences to the morphological expression of the pubic symphysis.

A linear mixed model was performed to analyze the relationship between estimated age and groups (i.e., Mexico, Puerto Rico). For this analysis, a Spanish reference sample, which is called a “parental group” was added to the analysis in order to test for estimated age differences among different geographical groups. A linear mixed effect model was also performed to test for asymmetry within the Mexican and Puerto Rican sample. After examining if significant differences or similarities were observed between samples, new age-at-death equations were created using linear regression analysis with an inverse calibration as proposed by Konigsberg and colleagues (1998). Several equations were evaluated by cross-validation analysis.

Linear mixed models: Asymmetry analysis

In this section, linear mixed models are used to separately examine the relationship between estimated age and sides on the Mexican and Puerto Rican samples. By performing the linear mixed model, it is of interest to know whether estimated age of the left and right sides differ, and if so, by how much.

<u>*Puerto Rico*</u>

When examining the relationship of estimated age using the Thin-Plate Splines/Bending Energy (TPS/BE) method and sides, a linear model in R was created. In this case, for a given variable (estimated age and/or sides) the t-statistic evaluates whether or not there is a significant association. Table 6-1 shows the analysis of variance for the aforementioned model and indicates that there is a significant main effect of estimated age using the TPS/BE method (F=17.243; *p*=6.307e-05). This means that overall, when we ignore which side is used, there is a significant variance in intercepts across ages estimated with the TPS/BE method. In contrast, the sides variable presented a non-significant value. This effect means that overall, the left and right sides do not present significant variance in intercept across individuals from the Puerto Rican sample.

The relationship between estimated age using the TPS/BE method and sides did not show a significant variance or interaction in intercept across individuals from Puerto Rico (F=0.1466, p=0.7025). Thus, the null hypothesis that side has no effect on estimated age is more likely, as the interaction results are not significant. When looking at the coefficients for the linear model in Table 6-2, these results further confirm that the regression slope from the left side is not statistically different from the right side (Figure 6-1).

Table 6-1. Analysis of variance for the linear model for the effects of estimated age with the TPS/BE method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
TPS/Bending Energy (TPS/BE)	1	5046	5045.9	17.243	6.307e-05*
Sides	1	101	100.8	0.3445	0.5584
TPS/Bending Energy: Sides	1	43	42.9	0.1466	0.7025
Residuals	116	33944	292.6		

Table 6-2. Parameter estimates for the effects of estimated age with the BE method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variables	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	11.485	10.286	1.116	0.2665
TPS/Bending Energy	0.890	0.3152	2.824	0.005*
Sides (Right)	-7.711	15.628	-0.493	0.622
TPS/Bending Energy:Sides(Right)	0.178	0.4652	0.3828	0.702

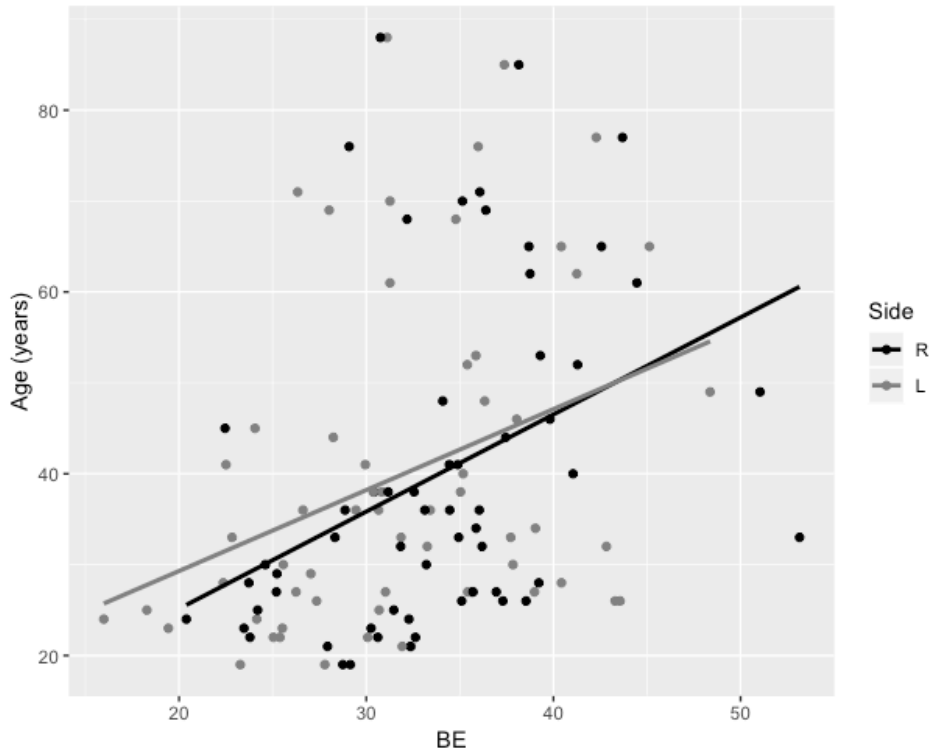

Figure 6-1. Chronological age versus the estimated age with the TPS/Bending Energy method and associated lm slopes for left and right sides, separately, on the Puerto Rican sample.

Similar results were observed when age was estimated with the Slice-Algee-Hewitt (SAH) Score method, the Ventral Curvature method (VC) + SAH score, and the VC + TPS/BE method using a linear mixed model. For all three methods, there is a significant main effect of estimated age, thus demonstrating that there is a significant variance in intercepts across ages estimated with all three methods. Similar to previous results, the sides variable presented a non-significant effect, and overall, left and right sides do not present a significant difference in intercepts across individuals from the Puerto Rican sample. Furthermore, all three computational methods demonstrated that the interaction between effect of estimated age and sides is not significant across all individuals. The VC method was the only method that did not have a significant effect on all scenarios, thus demonstrating that estimated age and sides do not show a significant difference in intercept across individuals, and that the interaction between effect of estimated age and sides is not significant. Overall, we can assume that sides have no effect on estimated age for all five computational methods for the Puerto Rican sample, and asymmetry should not be a confounding factor when creating new age-at-death equations (Table 6-3 to 6-10; Figure 6-2 to 6-5).

Mexico

Similar to the Puerto Rican sample, a lack of asymmetry was found when examining the relationship between estimated age on the left and right sides of the sample. In contrast to the Puerto Rican sample, age estimated by the TPS/BE method was the only variable that emerged as having a significant variance in intercept within individuals ($p=<0.02e-16$). The SAH score method, the VC method, the VC+ TPS/BE method, and the VC+SAH method did not significantly produce different variance in intercepts. When sides were examined, both left and

Table 6-3. Analysis of variance for the linear model for the effects of estimated age with the SAH method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variable	Df	SumSq	Mean Sq	F value	Pr(>F)
SAH Score (SAH)	1	10347.6	10347.6	42.0354	2.278e-09 *
Left/Right	1	186.4	186.4	0.7572	0.3860
SAH Score: Left/Right	1	45.0	45.0	0.1829	0.6697
Residuals	116	28554.9	246.2		

Table 6-4. Parameter estimates for the effects of estimated age with the SAH method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variable	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	5.303	7.681	0.690	0.491
SAH	0.835	0.179	4.664	8.350e-06*
Sides (Right)	1.870	10.652	0.175	0.860
SAH: Sides (Right)	-0.102	0.239	-0.427	0.669

Table 6-5. Analysis of variance for the linear model for the effects of estimated age with the VC method and left and right sides for the Puerto Rican sample.

Variable	Df	SumSq	Mean Sq	F value	Pr(>F)
Ventral Curvature (VC)	1	550	550.47	1.6700	0.1988
Left/Right	1	2	2.12	0.0064	0.9362
VC: Left/Right	1	345	345.13	1.0471	0.3083
Residuals	116	38236	329.62		

Table 6-6. Parameter estimates for the effects of estimated age with the VC method and left and right sides for the Puerto Rican sample.

Variable	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	20.23	12.372	1.635	0.104
VC	0.487	0.301	1.615	0.108
Sides (Right)	16.307	16.024	1.017	0.310
VC: Sides (Right)	-0.402	0.393	-1.0232	0.308

Table 6-7. Analysis of variance for the linear model for the effects of estimated age with the VC+SAH method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variable	Df	SumSq	Mean Sq	F value	Pr(>F)
VC+SAH	1	9524.9	9524.9	37.4907	1.288e-08*
Left/Right	1	56.8	56.8	0.2236	0.6372
VC+SAH: Left/Right	1	81.3	81.3	0.3201	0.5727
Residuals	116				

Table 6-8. Parameter estimates for the effects of estimated age with the VC+SAH method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variable	**Estimate**	**Std.Error**	**t value**	**Pr(>|t|)**
(Intercept)	9.364	9.573	0.978	0.330
VC+SAH	0.916	0.279	3.276	0.001*
Sides (Right)	9.429	13.950	0.675	0.500
VC+SAH: Sides (Right)	-0.301	0.402	-0.748	0.455

Table 6-9. Analysis of variance for the linear model for the effects of estimated age with the VC+ TPS/BE method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variable	Df	SumSq	Mean Sq	F value	Pr(>F)
VC+ TPS/BE	1	4367	4367.0	14.6478	0.0002105 *
Left/Right	1	17	16.6	0.0556	0.8140128
VC+ TPS/BE: Left/Right	1	167	167.1	0.5606	0.4555334
Residuals	116	34583	298.1		

Table 6-10. Parameter estimates for the effects of estimated age with the VC+ TPS/BE method and left and right sides for the Puerto Rican sample (* =significantly different with a α=0.05).

Variables	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	4.506	7.947	0.566	0.571
VC+ TPS/BE	0.855	0.185	4.606	1.059e-05*
Sides (Right)	4.682	11.106	0.421	0.674
VC+ TPS/BE: Sides (Right)	-0.143	0.254	-0.565	0.572

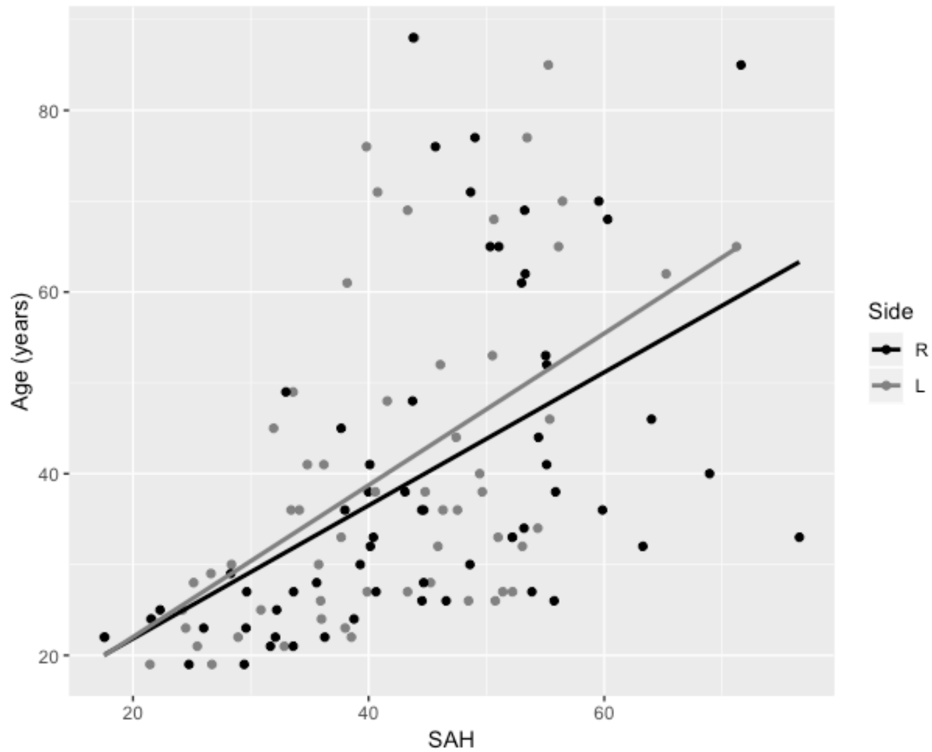

Figure 6-2. Chronological age versus the estimated age with the SAH Score method and associated lm slopes for left and right sides, separately, on the Puerto Rican sample.

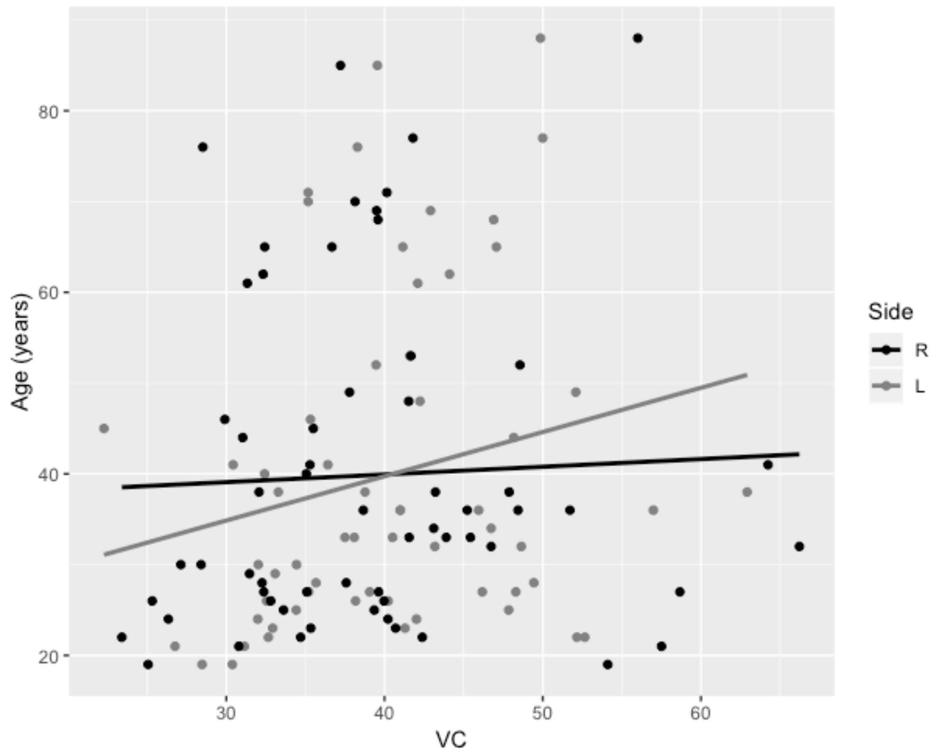

Figure 6-3. Chronological age versus the estimated age with the Ventral Curvature method and associated lm slopes for left and right sides, separately, on the Puerto Rican sample.

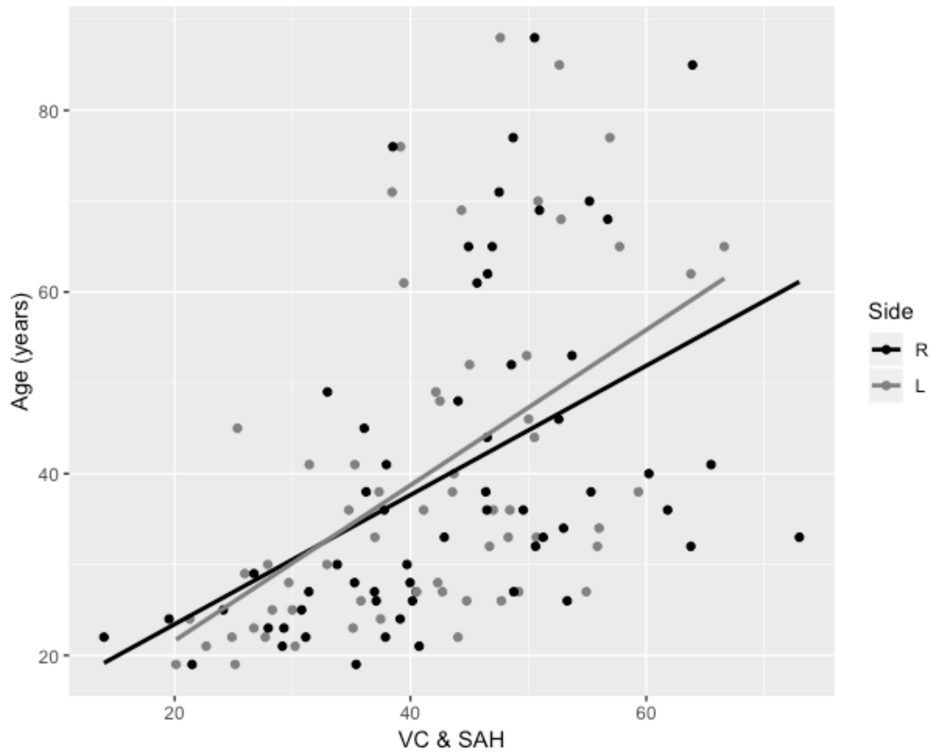

Figure 6-4. Chronological age versus the estimated age with the VC & SAH Score method and associated lm slopes for left and right sides, separately, on the Puerto Rican sample.

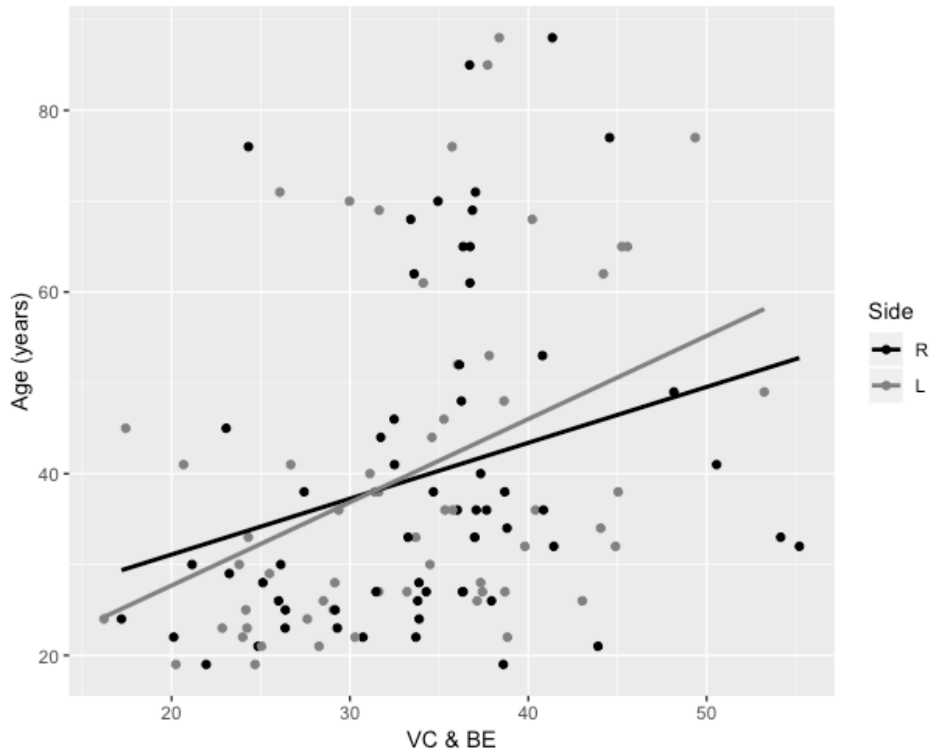

Figure 6-5. Chronological age versus the estimated age with the VC & TPS/BE Score method and associated lm slopes for left and right sides, separately, on the Puerto Rican sample.

right sides were non-significant for all five computational methods. Furthermore, the interaction between estimated age on all five methods and left and right sides was non-significant, demonstrating that effect of estimated age on chronological age did not differ when using left or right sides.

When examining the coefficients for the linear model, these results further confirm that the regression slope from the left side is not statistically different from the right side on all five computational models (Tables 6-11 to 6-19). Figure 6-6 to 6-10 show a scatterplot that displays the relationship between left and right sides for each computational method on the Mexican sample. The regression slopes for each side summarize the relationship between chronological age and estimated age. It is worth noting that all models present a positive relationship between chronological age and estimated age on both left and right sides.

Linear mixed models: Group analysis

Similar to the asymmetry analysis, a linear mixed model was used to examine the relationship between estimated age using all five computational methods in three different groups: Mexicans, Puerto Ricans, and Spaniards. As previously mentioned in the methods chapter, a third sample from Spain was included as a reference sample. Previous analysis of asymmetry of the Spanish sample shows similar results to the Mexican and Puerto Rican samples, demonstrating no significant interactions between estimated age and left and right sides. Thus, following standard practice, only the left side was included in the linear mixed model analysis.

Table 6-21 shows the analysis of variance for the model and indicates that there is a significant main effect for estimated age using the TPS/BE method ($F=34.46$, $p=1.381e-08$) and for the effect of groups ($F=4.29$, $p=0.014$); however, no interactions were found between both

Table 6-11. Analysis of variance for the linear model for the effects of estimated age with the TPS/BE method and left and right sides for the Mexican sample (* =significantly different with a α=0.05).

Variables	Df	SumSq	Mean Sq	F value	Pr(>F)
TPS/Bending Energy (TPS/BE)	1	16036.0	16036.0	237.1134	<2e-16*
Left/Right	1	0.8	0.8	0.0119	0.9139
TPS/Bending Energy: Left/Right	1	4.5	4.5	0.0665	0.7980
Residuals	36	2434.7	67.6		

Table 6-12.Parameter estimates for the effects of estimated age with the BE method and left and right sides for the Mexican sample.

Variables	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	24.950	24.358	1.024	0.312
TPS/Bending Energy	0.774	0.657	1.176	0.247
Sides (Right)	3.619	33.024	0.109	0.913
TPS/Bending Energy: Sides (Right)	-0.150	0.885	-0.170	0.865

Table 6-13. Analysis of variance for the linear model for the effects of estimated age with the SAH Score method and left and right sides for the Mexican sample.

Variable	**Df**	**SumSq**	**Mean Sq**	**F value**	**Pr(>F)**
SAH Score (SAH)	1	704.0	704.02	1.4358	0.2387
Left/Right	1	119.5	119.48	0.2437	0.6246
SAH Score: Left/Right	1	0.0	0.02	0.0000	0.9952
Residuals	36	17652.5	490.35		

Table 6-14. Parameter estimates for the effects of estimated age with the SAH method and left and right sides for the Mexican sample.

Predictors	**Estimate**	**Std.Error**	**t value**	**Pr(>|t|)**
(Intercept)	38.269	20.441	1.872	0.069
SAH	0.402	0.540	0.744	0.461
Sides (Right)	-3.693	26.614	-0.138	0.890
SAH: Sides (Right)	0.004	0.668	0.005	0.995

Table 6-15. Analysis of variance for the linear model for the effects of estimated age with the VC method and left and right sides for the Mexican sample.

Variables	Df	SumSq	Mean Sq	F value	Pr(>F)
Ventral Curvature (VC)	1	297.0	297.03	0.6199	0.4362
Left/Right	1	35.1	35.10	0.0733	0.7882
VC: Left/Right	1	893.8	893.78	1.8653	0.1805
Residuals	36	17250.1	479.17		

Table 6-16. Parameter estimates for the effects of estimated age with the VC method and left and right sides for the Mexican sample.

Variables	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	20.714	21.616	0.958	0.344
VC	0.798	0.520	1.533	0.133
Sides (Right)	38.943	30.68	1.269	0.212
VC: Sides (Right)	-0.997	0.730	-1.365	0.180

Table 6-17. Analysis of variance for the linear model for the effects of estimated age with the VC+SAH Score method and left and right sides for the Mexican sample.

Variables	**Df**	**SumSq**	**Mean Sq**	**F value**	**Pr(>F)**
VC+SAH	1	297.0	297.03	0.6199	0.4362
Left/Right	1	35.1	35.10	0.0733	0.788
VC+SAH: Left/Right	1	893.8	893.78	1.8653	0.180
Residuals	36	17250.1	479.17		

Table 6-18. Parameter estimates for the effects of estimated age with the VC+SAH method and left and right sides for the Mexican sample.

Variables	**Estimate**	**Std.Error**	**t value**	**Pr(>|t|)**
(Intercept)	29.278	18.258	1.603	0.117
VC+SAH	0.646	0.479	1.348	0.185
Sides (Right)	7.024	25.787	0.272	0.786
VC+SAH: Sides (Right)	-0.275	0.643	-0.427	0.671

Table 6-19. Analysis of variance for the linear model for the effects of estimated age with the VC+ TPS/BE Score method and left and right sides for the Mexican sample.

Variables	Df	SumSq	Mean Sq	F value	Pr(>F)
VC+ TPS/BE	1	868.8	868.84	1.8007	0.1880
Left/Right	1	33.8	33.76	0.0700	0.7929
VC+ TPS/BE: Left/Right	1	203.2	203.24	0.4212	0.5205
Residuals	36	17370.1	482.50		

Table 6-20. Parameter estimates for the effects of estimated age with the VC+ TPS/BE method and left and right sides for the Mexican sample.

Variables	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	24.153	21.204	1.139	0.262
VC+ TPS/BE	0.752	0.538	1.398	0.170
Sides (Right)	16.754	29.483	0.568	0.573
VC+ TPS/BE: Sides (Right)	-0.481	0.742	-0.649	0.520

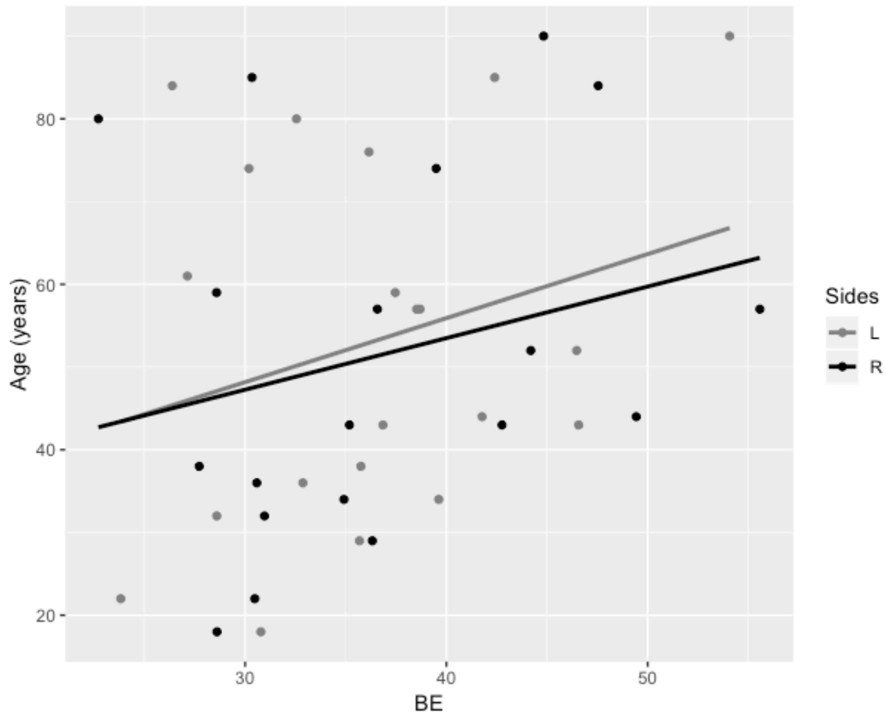

Figure 6-6. Chronological age versus the estimated age with the TPS/Bending Energy method and associated lm slopes for left and right side, separately, on the Mexican sample.

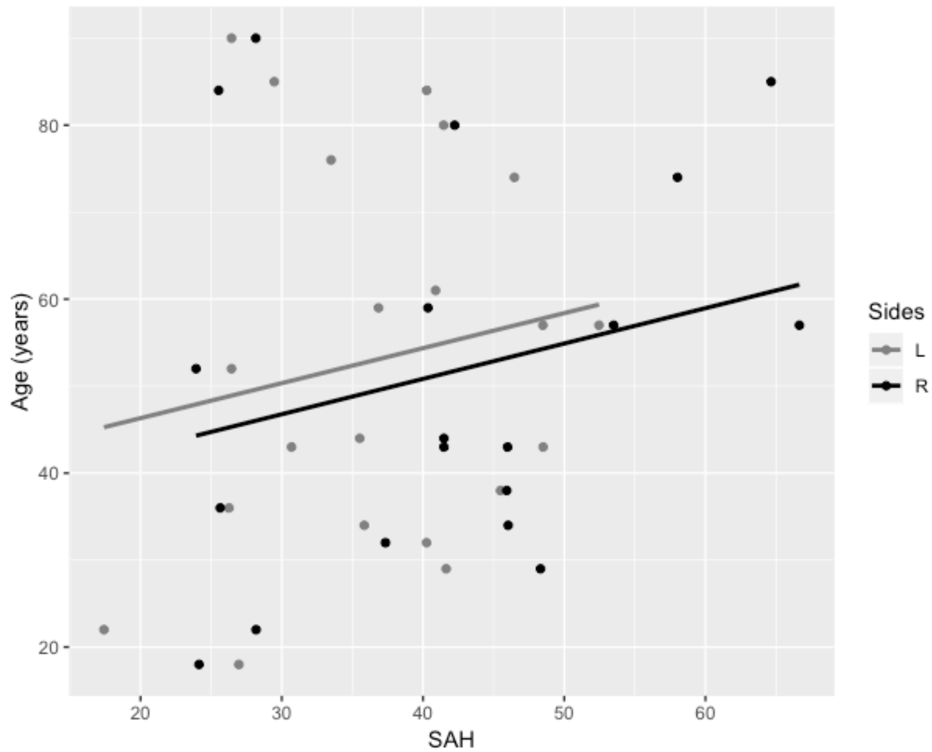

Figure 6-7. Chronological age versus the estimated age with the SAH Score method and associated lm slopes for left and right side, separately, on the Mexican sample.

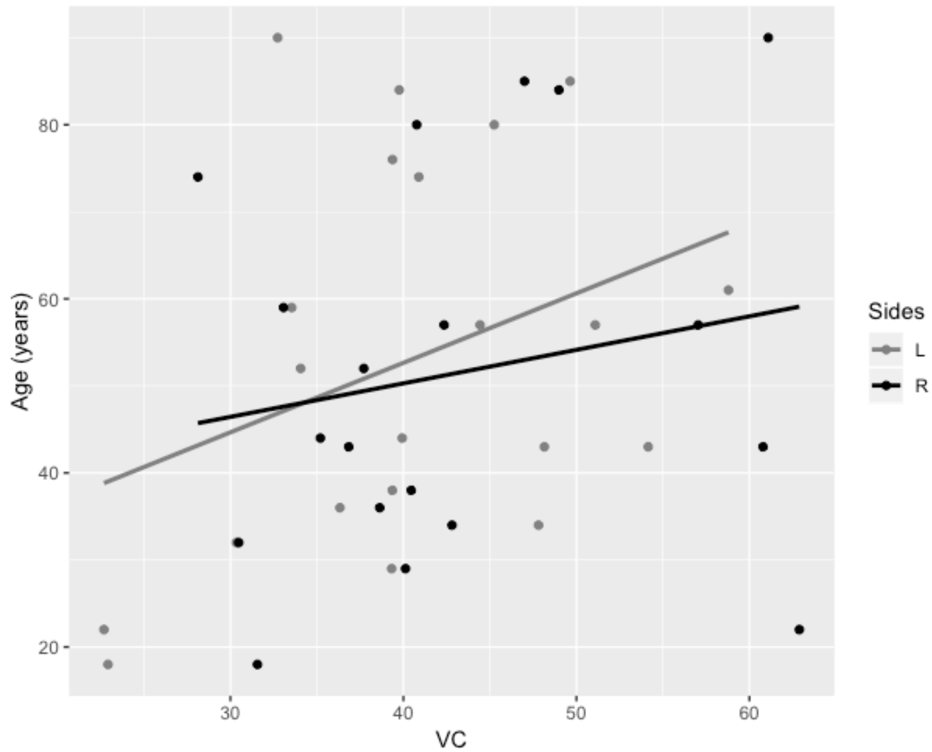

Figure 6-8. Chronological age versus the estimated age with the Ventral Curvature method and associated lm slopes for left and right sides, separately, on the Mexican sample.

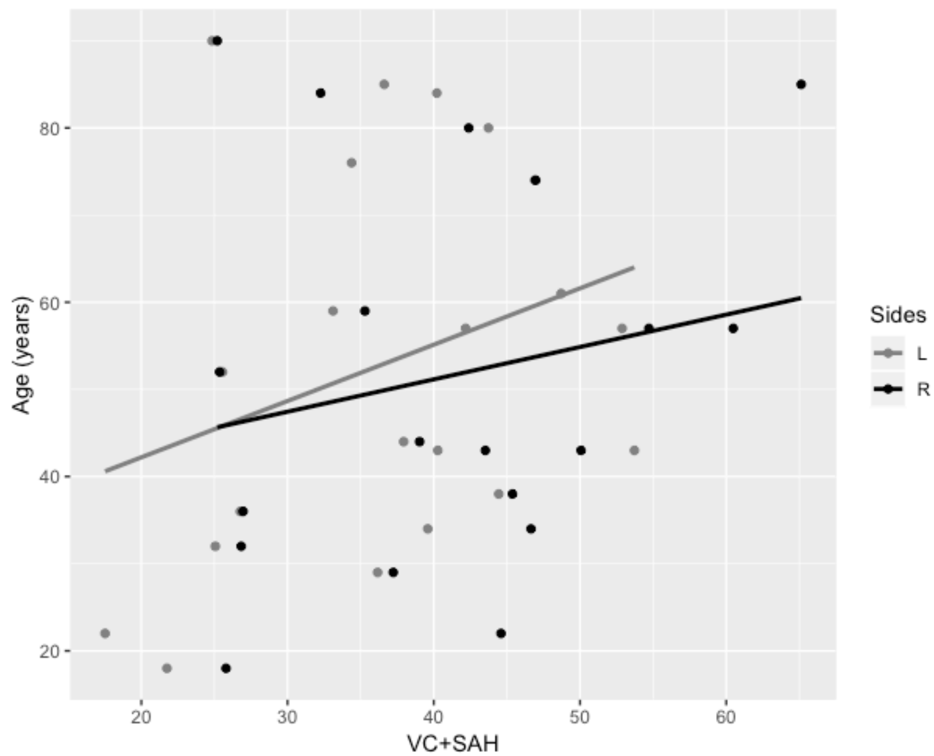

Figure 6-9. Chronological age versus the estimated age with the VC & SAH Score method and associated lm slopes for left and right sides, separately, on the Mexican sample.

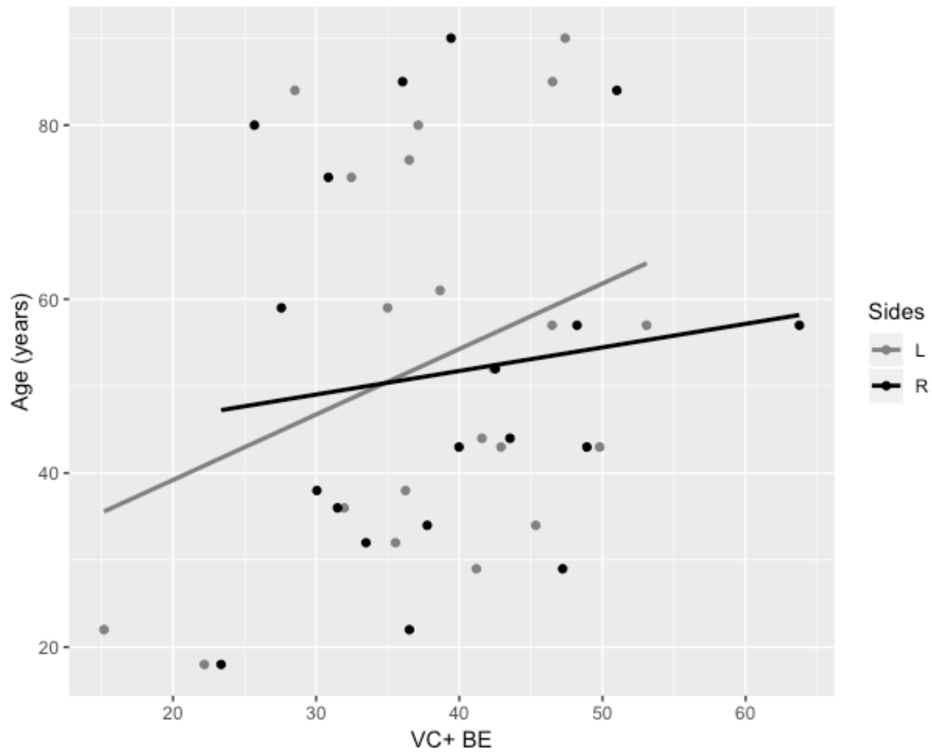

Figure 6-10. Chronological age versus the estimated age with the VC & TPS/BE method and associated lm slopes for left and right sides, separately, on the Mexican sample.

Table 6-21. Analysis of variance for the linear model for the effects of estimated age with the SAH method and all three groups age with the TPS/BE method and groups.

Variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
TPS/Bending Energy (BE)	1	7387.49	7387.49	34.462	1.381e-08*
Groups	2	1841.26	920.63	4.294	0.014*
TPS/BE:Groups	2	32.28	16.14	0.075	0.927
Residuals	249	53375.75	214.36		

variables. The significance of both variables means that the estimated age and groups present significant differences within each sample, but the relationship between estimated age and groups does not present a significant variance in intercept, and the slopes for each group are similar. Thus, the null hypothesis that groups have no effect on estimated age is accepted. The coefficients for the linear mixed model confirm that the regression slope for all three groups is not statistically different from each other (Table 6-22, Figure 6-11).

Similar results were obtained when examining the relationship between the VC method, the VC+SAH method, and the VC+ TPS/BE method and all three groups. For all three methods, there was a significant main effect for estimated age and for the effect of groups; however, no interactions were found between both variables. When examining the coefficients for all three variables, the interactions between estimated age and groups showed that the regression lines are Table 6-21. Analysis of variance for the linear model for the effects of estimated not statistically different (Figures 6-12 through 6-15). Tables 6-23 through 6-30 display the results of the coefficients for all three analysis.

The SAH score was the only analysis that showed a significant main effect for the estimated age, but a non-significant main effect for the group variable. Similar to previous analysis, no interactions were found between both variables. Thus, the null hypothesis that groups have no effect on estimated age is rejected.

New regression equation models

New regression models for all five computational methods were built using inverse calibration (Konigsberg and Frankenberg 1997) after log transformation was applied to the score variable. Log transformation was applied to one of the variables (score) in an attempt to make the age

Table 6-22. Parameter estimates for the effects of estimated age with the BE method and groups.

Variables	**Estimate**	**Std.Error**	**t value**	**Pr(>|t|)**
(Intercept)	20.549	5.479	3.750	0.0002*
TPS/Bending Energy	0.764	0.189	4.039	7.1399e-05*
Spain:Mexico	4.400	17.186	0.256	0.7981
Spain:Puerto Rico	-9.06	10.369	-0.874	0.3829
TPS/BE:Spain:Mexico	0.009	0.479	0.019	0.9844
TPS/BE:Spain:Puerto Rico	0.125	0.329	0.381	0.7028

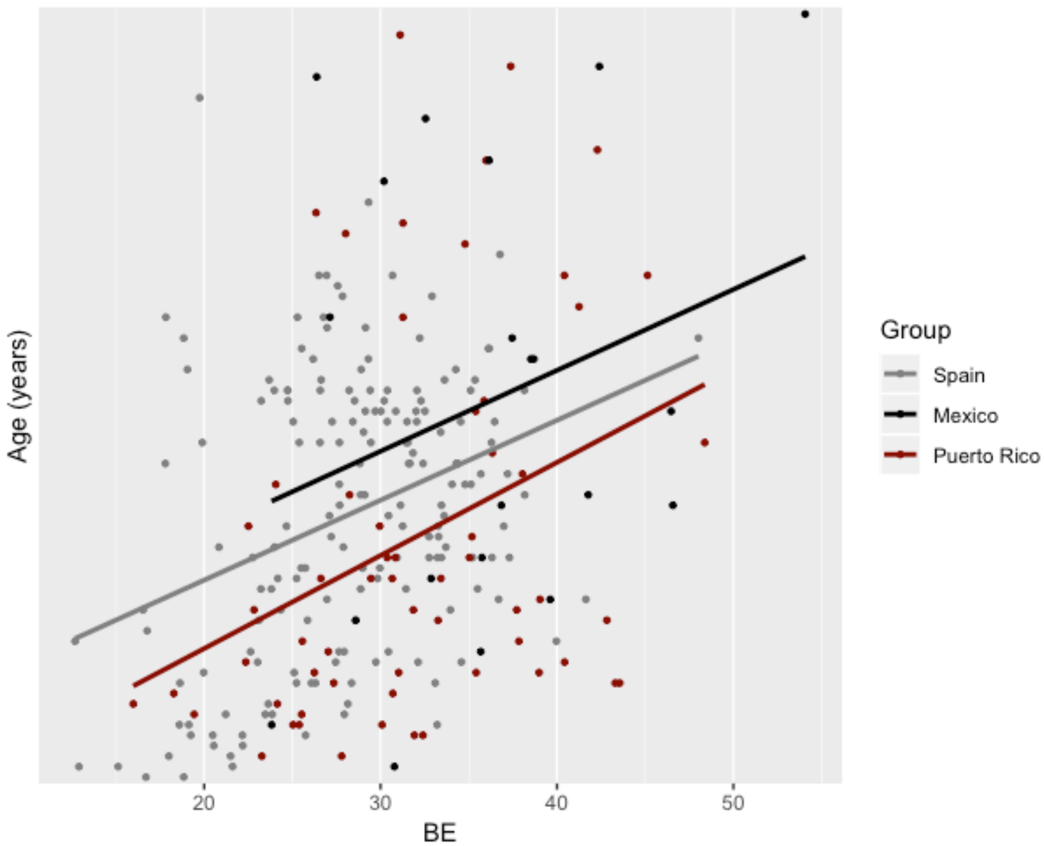

Figure 6-11. Chronological age versus the estimated age with the TPS/BE method and associated lm slopes for each group, Mexico, Puerto Rico and Spain.

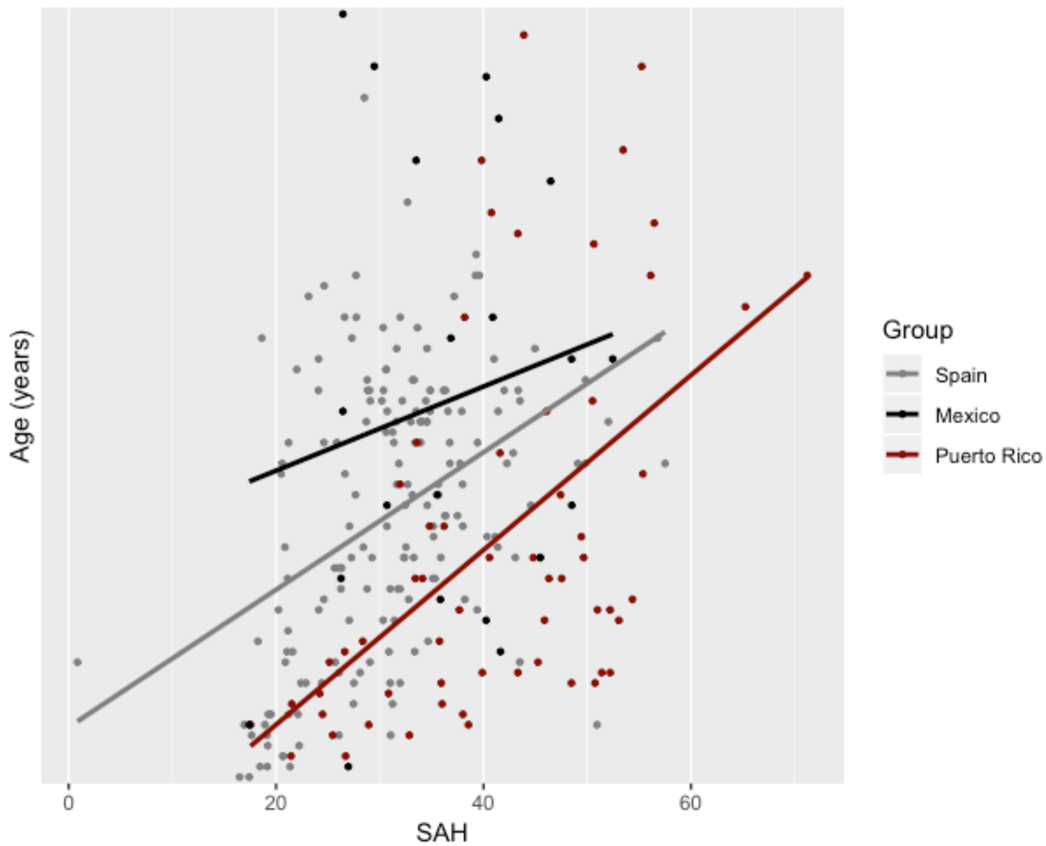

Figure 6-12. Chronological age versus the estimated age with the SAH method and associated lm slopes for each group, Mexico, Puerto Rico and Spain.

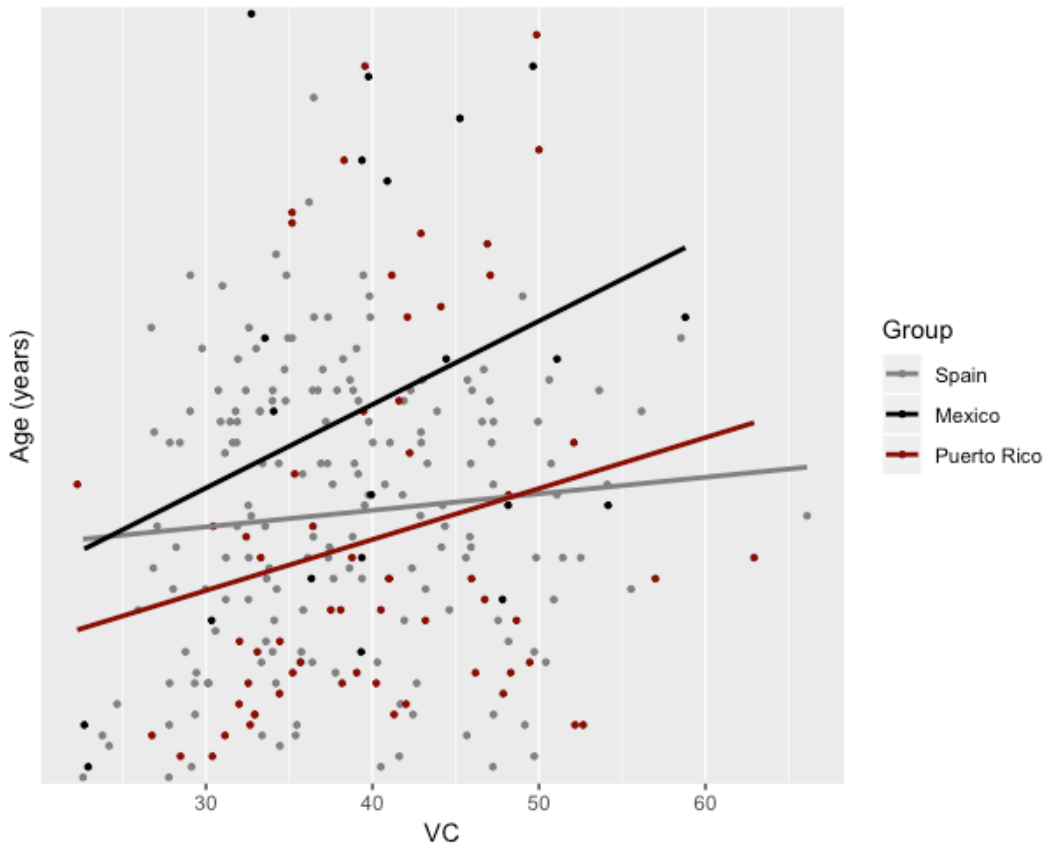

Figure 6-13. Chronological age versus the estimated age with the VC method and associated lm slopes for each group, Mexico, Puerto Rico and Spain.

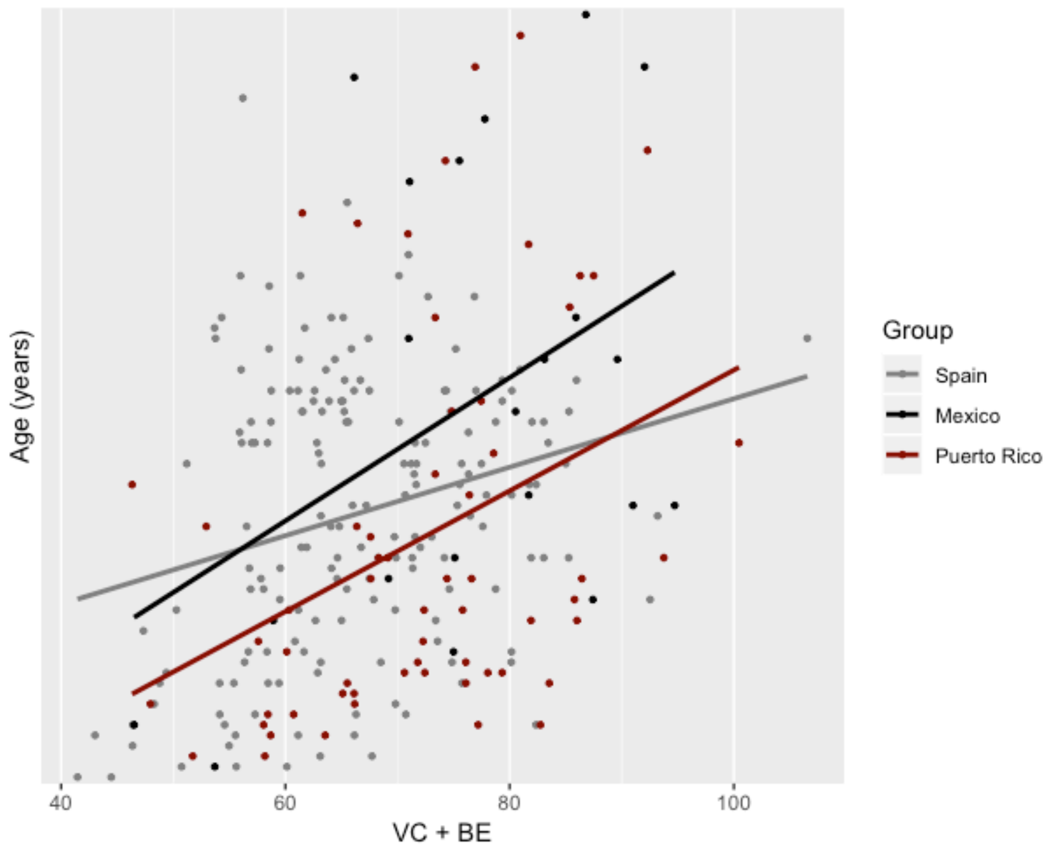

Figure 6-14. Chronological age versus the estimated age with the VC + TPS/BE method and associated lm slopes for each group, Mexico, Puerto Rico and Spain.

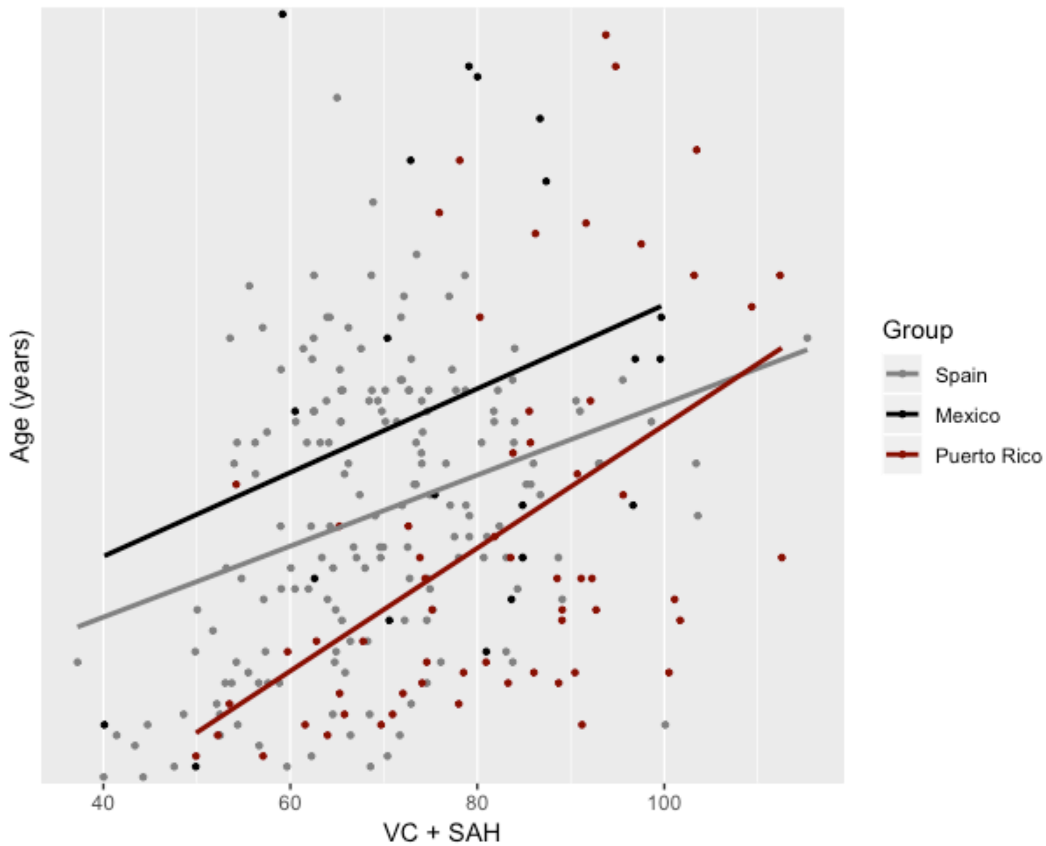

Figure 6-15. Chronological age versus the estimated age with the VC + SAH method and associated lm slopes for each group, Mexico, Puerto Rico and Spain.

Table 6-23. Analysis of variance for the linear model for the effects of estimated age with the SAH method and all three groups.

Variables	Df	SumSq	Mean Sq	F value	Pr(>F)
Slice-Algee-Hewitt Score (SAH)	1	8155.26	8155.26	41.45	6.15e-07*
Groups	2	5188.83	2594.41	13.18	3.59
SAH:Groups	2	311.56	155.78	0.79	0.45
Residuals	249	48981.137	196.71	NA	NA

Table 6-24. Parameter estimates for the effects of estimated age with the SAH method and groups.

Variables	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	21.77	4.09	5.317	2.33e-07
SAH	0.65	0.12	5.17	4.74e-07
Spain:Mexico	16.49	13.57	1.21	0.225
Spain:Puerto Rico	-16.46	7.99	-2.05	0.040
SAH:Spain:Mexico	-0.25	0.36	-0.69	0.485
SAH:Spain:Puerto Rico	0.17	0.20	0.87	0.384

Table 6-25. Analysis of variance for the linear model for the effects of estimated age with the VC method and all three groups.

Variables	**Df**	**SumSq**	**Mean Sq**	**F value**	**Pr(>F)**
Ventral Curvature (VC)	1	1556.23	1556.23	6.72	0.010*
Group	2	2681.28	1340.64	5.79	0.003*
VC:Spain	2	775.51	387.75	1.67	0.189
Residuals	249	57623.76	231.42		

Table 6-26. Parameter estimates for the effects of estimated age with the VC method and groups (* =significantly different with a α=0.05).

Variables	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	36.19	5.79	6.24	1.79e-09*
VC	0.15	0.14	1.06	0.289
Spain:Mexico	-15.48	16.10	-0.96	0.337
Spain:Puerto Rico	-15.96	11.87	-1.34	0.180
VC:Spain:Mexico	0.64	0.39	1.63	0.103
VC:Spain:Puerto Rico	0.32	0.29	1.12	0.262

Table 6-27. Analysis of variance for the linear model for the effects of estimated age with the VC+ TPS/BE method and all three groups (* =significantly different with a α=0.05).

Variables	**Df**	**SumSq**	**Mean Sq**	**F value**	**Pr(>F)**
VC+ TPS/BE	1	7314.87	7314.87	34.30	1.481e-08*
Group	2	1848.10	924.05	4.33	0.014*
VC+ TPS/BE:Group	2	383.68	191.84	0.89	0.407
Residuals	249	53090.13	213.21		

Table 6-28. Parameter estimates for the effects of estimated age with the VC+ TPS/BE method and groups (* =significantly different with a α=0.05).

Variables	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	26.33	4.65	5.66	4.120e-08*
VC+ TPS/BE	0.54	0.15	3.51	0.000*
Spain: Mexico	-2.17	14.84	-0.14	0.883
Spain:Puerto Rico	-16.96	9.33	-1.81	0.070
VC+ TPS/BE:Spain:Mexico	0.20	0.38	0.53	0.592
VC+ TPS/BE:Spain: Puerto Rico	0.37	0.28	1.31	0.189

Table 6-29. Analysis of variance for the linear model for the effects of estimated age with the VC+SAH method and all three groups (* =significantly different with a α=0.05).

Variables	**Df**	**SumSq**	**Mean Sq**	**F value**	**Pr(>F)**
VCSAH	1	7985.29	7985.29	40.27	1.036e-09*
Spain	2	4964.72	2482.36	12.51	6.593e-06*
VCSAH:Spain	2	315.53	157.76	0.79	0.452
Residuals	249	49371.24	198.27		

Table 6-30. Parameter estimates for the effects of estimated age with the VC+BE method and groups (* =significantly different with a α=0.05).

Variables	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	23.41	4.20	5.56	6.654e-08*
VCSAH	0.59	0.12	4.62	6.035e-06*
Spain:Mexico	5.86	12.48	0.46	0.638
Spain:Puerto Rico	-18.90	8.18	-2.31	0.021*
VCSAH:SpainMexico	0.051	0.33	0.15	0.876
VCSAH:Spain:Puerto Rico	0.261	0.20	1.25	0.211

variable homoscedastic (same variance around the regression line for x variable) with respect to the score variable. The results of the regression analysis for all three samples is included in Table 5-49. Results were quite similar for all three methods, with RMSE ranging from 14.98 to 15.71. The log transformation of the score did not produce significant improvements and the results of the raw score versus age produced similar R^2 results (Table 6-31).

Because the sample size from the Mexican group was quite small, it was removed from the regression analysis and used for cross validation analysis. Similar to previous analysis, a log transformation was applied to one variable (score) in an attempt to make the age variable homoscedastic with respect to the score variable. The results of the regression analysis and their respective graphs are included in Table 6-32 and Figure 6-16. When the Mexican sample was removed from the regression analysis, the RMSE improved significantly. For the TPS/Bending Energy method, the RMSE decreased from 15.00 years to 14.02 with the raw Age vs Score model, and with a log transformation, it decreased from 14.98 to 14.17. For the SAH score the RMSE values decreased from 15.76 to 13.48 for raw Age vs Score method and from 15.25 to 13.61 with a log transformation. When the VC method was analyzed, the RMSE decreased from 15.71 to 14.61 with the raw Age vs Score method and increased from 15.41 to 14.53 with a log transformation.

R^2 remained significantly low for the regression analysis of Puerto Ricans and Spanish groups combined. The only significant improvement in R^2 was for the SAH score, which improved from 0.3 to .16 for raw scores. Because the log transformation did not provide an improvement to the models, the regression of raw score into raw age will be used for cross validation analysis with the Mexican sample.

Table 6-31. Summary of the results for the TPS/Bending Energy method, the SAH Score method, and the Ventral Curvature Method used in univariate regression models for the pooled sample (Mexico, Puerto Rico, and Spain) of the left side (* =significantly different with a α=0.05).

Pooled sample				
Thin-Plate Splines/Bending Energy Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	<0.0001*	0.09	15.00	AGE = 48.732772 - 2.8741794*TPS/BEScore
Age vs log(Score)	<0.0001*	0.09	14.98	AGE = 46.713853 - 17.242095*logTPS/BEScore
SAH Score Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	0.758	0.03	15.76	AGE= 42.985822 - 2.0155397*SAHScore
Age vs log(Score)	<0.0001*	0.03	15.25	AGE = 28.499029 - 19.663995*logSAHScore
Ventral Curvature Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	.186	0.06	15.71	AGE = 40.418148 + 24.821881*VCScore
Age vs log(Score)	<0.0001*	0.04	15.41	AGE = 58.653776 + 14.436573*logVCScore

Table 6-32. Summary of the results of the TPS/Bending Energy method, the SAH Score method, and the Ventral Curvature Method used in univariate regression models for the Puerto Rican sand Spanish sample, with left only (* =significantly different with a α=0.05)

Puerto Rico + Spain				
Thin-Plate Splines/Bending Energy Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	<0.0001*	.09	14.02	AGE = 48.752059 - 3.342246* TPS/BEScore
Age vs log(Score)	<0.0001*	.09	14.17	AGE = 45.858116 - 16.50479*log TPS/BEScore
SAH Score Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	<0.0001*	.16	13.48	AGE= 58.62323 - 85.470048*SAHScore
Age vs log(Score)	<0.0001*	.14	13.61	AGE = 14.037731 - 37.934785*logSAHScore
Ventral Curvature Method				
Transformation	**p-value**	**R^2**	**RMSE**	**Equation**
Age vs Score	0.032*	.01	14.61	AGE = 35.871585 + 69.039904*VCScore
Age vs log(Score)	0.006*	.03	14.53	AGE = 55.865936 + 5.5405596*logVCScore

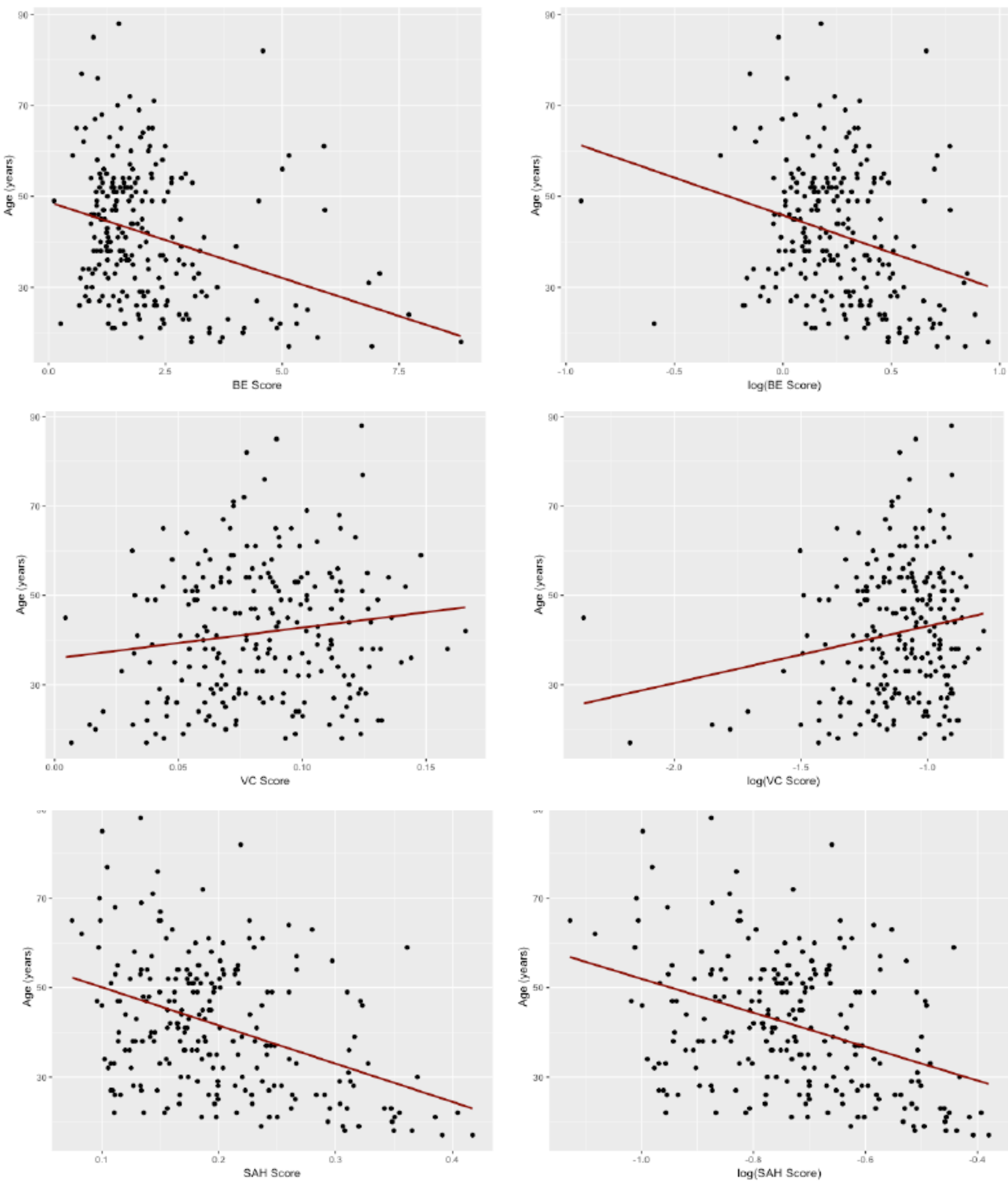

Figure 6-16. Regression slopes for all three computation methods, including raw score vs raw age and raw age vs log of score.

When bias and inaccuracies were examined for the Mexican sample on the new three equations, the SAH method produced the least bias (-6.09) and the smallest inaccuracy with 16.81 years. This was followed by the TPS/BE method with a bias of -8.49, and inaccuracies of 18.96 years. Finally, the VC method produced the greatest bias with -8.75 and inaccuracies as high as 20.52. When the bias values are plotted against years for all three methods, it can be seen that positive bias values (overestimation) are seen for individuals below 40 years of age, and negative values (underestimation) are seen for individuals above 50 to 60 years of age (Table 6-33 and Figure 6-17).

The following chapter will discuss in further detail the results presented in this chapter. In addition, the results will be discussed in relationship to previous findings in the literature. Finally, several recommendations are provided as to how to utilize the newly developed equations, and a thorough discussion of the limitations encountered during this study.

Table 6-33. Bias and inaccuracies for the new regression equations using the Mexican sample.

	TPS/Bending Energy method	**Slice-Algee-Hewitt method**	**Ventral Curvature method**
Bias	-8.49	-6.09	-8.75
Inaccuracy	18.96	16.81	20.52

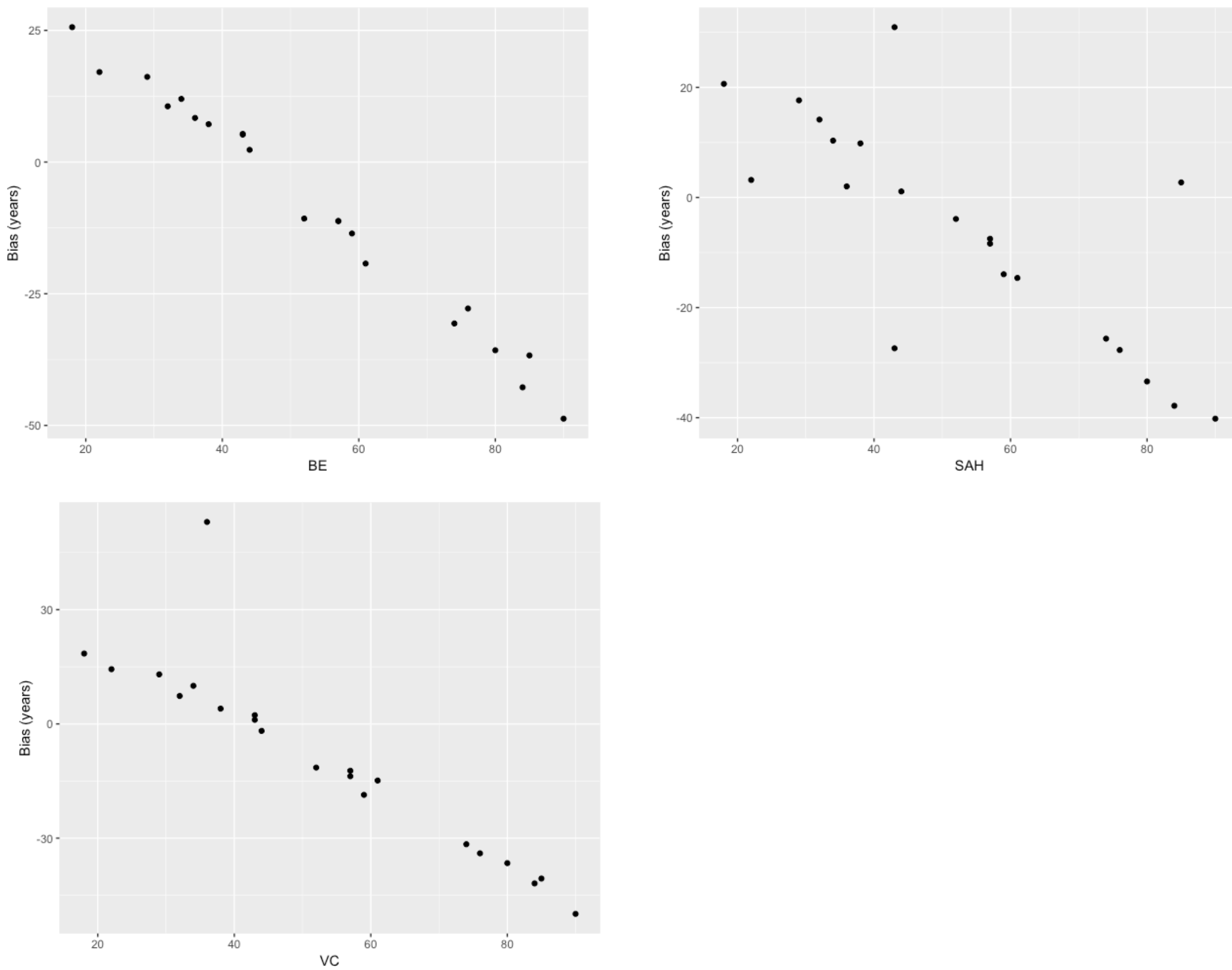

Figure 6-17. Bias in years for the new regression equations using the TPS/BE method, the SAH score method, and the VC method on the Mexican sample.

CHAPTER SEVEN
DISCUSSION

The purpose of this chapter is to discuss the hypothesis and results outlined in Chapters Three and Four. The goal of this study was twofold. First, I examine how well some current age-at-death techniques of the pubic symphysis yield accurate estimates for several groups in Latin America. Several aging techniques were included in this analysis, including the Suchey Brooks method and five newly developed computational aging methods using 3D laser scans, created by Slice and Algee-Hewitt (2015), and Stoyanova and colleagues (2015, 2018). A second goal of the study was to address the question concerning population specificity in aging techniques within groups from Latin America. By creating new statistical models and linear regression equations for samples originating from Latin America, population specificity in age-at-death techniques is discussed and future recommendations when attempting to estimate age on groups from a Hispanic background in a forensic context are presented.

Are current aging techniques appropriate for aging populations from Latin America?

The majority of the methods utilized to estimate age-at-death are based on the premise that progressive changes in bone morphology reflect degenerative processes in the adult skeleton, making them a good indicator of age (Kemkes-Grottenthaler 2002). However, many of these degenerative processes can vary among populations and within individuals due to differences in growth, development, and lifestyle (Baccino et al. 1994; Kemkes-Grottenthaler 2002; Schmitt 2002). As many of the aging techniques utilized in anthropology were developed with individuals who are genetically and morphologically different from individuals of Latin American origin, it is imperative to examine how well these methods perform on samples originating from Latin America, and if they are appropriate to use in a forensic setting.

Overall, results from this study suggest that traditional and computational aging techniques utilizing the pubic symphysis perform reasonably well for individuals between 35 to 45 years of age for samples of Mexican and Puerto Rican origin. However, the magnitude of error for younger (< 35 years of age) and older individuals (> 45 years of age) was higher when using the Suchey Brooks method. Overall, all computational methods performed slightly better on these younger and older individuals. Even though the performance of the computational methods was better in older individuals when compared to the traditional method, the directionality and magnitude of error was still significantly high. Results further indicate that the magnitude and directionality of error increase as chronological age increased for all methods. Furthermore, a trend to overestimate younger individuals and underestimate older individuals was seen in all methods.

When exploring the questions concerning population specificity, newly created computational equations for Latin American samples did not necessarily improve age estimates. Even though RMSE levels for new computational equations improved when using the new samples from Latin America and Spain, and the magnitude and directionality of error improved slightly, the improvements are more likely associated with the size and distribution of the reference sample rather than population variation.

Suchey-Brooks method

When comparing traditional and computational methods, the Suchey-Brooks method performs fairly well when estimating age-at-death on middle aged (mid 30's to late 40s) individuals for the pooled sample, as well as Mexico and Puerto Rico separately. On average, 44% or higher of estimated ages fall within 5 years or less of the chronological age. Higher percentages were observed when the sample was separated by groups. Correlations values between chronological age and estimated age were significantly higher when compared to all five computational methods. When the Suchey-Brooks method was applied to the Puerto Rican sample, inaccuracies and bias levels were the lowest. In contrast, applying the Suchey-Brooks method to the Mexican sample presented the highest bias and inaccuracy values in comparison to the Puerto Rican sample. These results can be explained by several factors, including the age distribution of the reference and test sample, and the calculation of bias and inaccuracy from the mean ages that originate from the age distribution.

First, previous publications have shown that the Suchey Brooks method tends to be more accurate when aging younger individuals than the elderly (Miranker 2016; Rissech et al. 2011; Hens et al. 2008). The Suchey Brooks method is a regression-based model in which the test sample tends to mimic the age distribution of the reference sample used to create the method. This trend is due to the nature of the reference sample of the method, which has far more young adults (<45 years of age) than older adults (45 to 90 years of age). If younger adults are the only ones well represented in a reference sample, the method will have lower mean ages for each phase, disregarding potential informative age-related changes within older individuals. This means that the mean age for each phase on the Suchey Brooks method is skewed towards younger individuals, and consequently, lower mean ages. Second, this issue has the potential of increasing bias and inaccuracy for the age groups that are underrepresented in the reference sample. For this study, the Puerto Rican sample has a higher number of younger individuals (<40 years of age with a mean age of 39.87) than does the Mexican sample, accounting for the generally good performance of the Suchey Brooks method in the Puerto Rican sample. Simply said, the age distribution of the Puerto Rican sample is more similar to the Suchey Brooks reference sample than the Mexican sample. The high inaccuracy and bias values that resulted from using the Suchey Brooks method on the Mexican sample can be explained by the aforementioned trends, as the Mexican sample is composed of individuals over 50 years of age, with a mean age of 53.05. Individuals above the age of 50 years of age are underrepresented in the reference sample. It is expected for bias and inaccuracy to be lower when the Suchey Brooks method is tested on a population with a similar age-at-death structure than the reference sample.

When results from this study are compared to previously published studies, it is surprising to note that the directionality and magnitude of error presented between estimated mean age and chronological age in this study are much lower than for other studies. Miranker (2016) has shown that on a sample of 131 White males from the William M. Bass Donated Collection, the Suchey-Brooks method presented bias levels as high as -20.01 and measures of inaccuracy of 20.3 years. It is worth noting that the highest bias and inaccuracy levels found in the current analysis for the Suchey-Brooks method are -9.27 and 13.07 years, respectively, in the Mexican group alone. Schmitt (2004) presented bias levels of -14.5 and inaccuracies measures of 17.2 years for a small sample (n=37) of Thai males using the Suchey-Brooks method. In contrast, when a large (n=202) Italian sample was analyzed by Hens and colleagues (2008), levels of bias

(-11.1) and inaccuracies (13.6 years) were comparable to values presented in this study. Although different levels of bias and inaccuracies have been reported for other populations across the world, trends of overestimating individuals younger than 40 years of age and underestimating older individuals were similar across all studies. Furthermore, all previous studies, including the current study, showed that the average estimation of error increased with the progression of chronological age.

So, why is there so much variability in the directionality and magnitude of error between studies when using the Suchey-Brooks method? Prior studies have suggested that such variability might be attributed to several factors including secular change and interpopulation variability. First, several studies have demonstrated that significant secular change trends are apparent in the onset of skeletal maturation (Hoppa 2000, Langey-Shirley and Jantz 2010, Meadow and Jantz 1995; Malina 1975). For example, Hoppa (2000) demonstrated that age related changes of the pubic symphysis are significantly different when applying the Suchey-Brooks method to a forensic sample belonging to the Dade County Medical Examiner's Office with birth years belonging to the 20th century, and an archeological sample from the 18th-19th century. Hoppa (2002) was able to demonstrate a differential timing of age-related changes in the pubic symphysis, with noticeable differences in the rate of early development and late degeneration between all samples. Other studies have demonstrated similar results using a forensic sample of 82 males from India. Sinha and Gupta (1995) found significant differences in the mean age of several phases when using the Todd aging phase-system, with lower mean ages of development on phases II, II, and IV. Similar results were observed when the sample was aged with the Mckern and Stewart method (Sinha and Gupta 1995). Thus, it is possible to suggest that the

Suchey-Brooks reference sample is not representative of late 20th – early 21st century populations.

Studies examining other skeletal features had demonstrated that secular change is a variable to consider when aging a skeleton. For example, Langley-Shirley and Jantz (2010) have shown that a modern American sample of clavicles fuses approximately four years earlier than a sample of early 20th century Americans, and 3.5 years earlier than a sample of Korean War era Americans. The authors attribute the skeletal aging acceleration to improvements in health, life expectancy, and socioeconomic status rather than "ethnicity" differences. Aside from secular change, interpopulation variability has been reported as an influential variable when presented with mean age differences between populations.

Interpopulation variability refers to differences in the factors affecting growth and development between and within populations. Within and between populations, there is variation regarding access to environmental conditions (i.e., health services, education, good nutrition) affecting growth and development. This variation is largely due to political, economic, and social circumstances that influence exposure to disease, access to quality health care, and the ability to obtain nutritious food. Schmeling and colleagues (2000) have suggested that the rate of ossification in age groups between 0 to 20 years of age is primarily affected by the socioeconomic development of a population. Schmeling et al. (2000) was able to analyze over 80 publications on skeletal maturation in populations from across the world, and results suggest that skeletal maturation takes place in identical and defined stages. In a different study, Schmeling and colleagues demonstrated via regression analysis that medical and economic development (i.e., per capital income and life expectancy at birth as parameters of modernization) are positively correlated with ossification rates. Thus, when conditions for growth are not optimal, growth is strongly influenced by the environmental conditions a child is exposed to. Examples of these environmental circumstances affecting growth and development can be seen in studies examining migrant and non-migrant populations. Lasker and Evans (1961) compared anthropometric measurements in migrants and non-migrants from Michoacán, Mexico. They examined whether or not there are significant differences in the mean dimensions in upper and lower limb dimensions between 1) individuals born in and who have always lived in Michoacán, 2) individuals born in Michoacán but who migrated to the United States at some point in their lives, 3) and individuals living in Michoacán who were born in the United States of Mexican parents. One of the significant differences found between the samples was that the migrant sample from the United States who returned to Michoacán shows longer upper limbs in males compared to the other two samples (Lasker and Evans 1961). Lasker and Evans suggest that the impact of a better socioeconomic progress and modernization in medicine in the United States is a potential factor for the anthropometric differences between populations.

In a similar study, Bogin et al. (1997) conducted research between Mayan populations that migrated to the United States and a Mayan population that lived in a rural village in Guatemala. They observed that families that invested economic and social resources in their children tended to have taller children. They state that children who continue to live in the rural villages are shorter on average than the Guatemalan children who migrated to the United States. Therefore, it is possible to assume that secular change and interpopulation variation observed between the results from this study and results from previously published work may represent secular trends between samples and/or differences in lifestyle between groups.

Computational methods

As previously mentioned, all five computational methods (i.e., SAH score method, TPS/BE method, VC method, TPS/BE+VC, and SAH+VC method) performed fairly well on the Latin American sample. Grand means calculated across all five methods show that 29% of the pooled sample falls within a 5 year range from the chronological age, while 50% of the pooled sample falls within 10 years or less. Furthermore, it can be seen that the direction of error for all computational methods tended to overestimate ages of young individuals, and underestimated ages for individuals over 60 years of age. This trend is similar to the one seen when using the Suchey-Brooks method, and this was also consistently seen when the sample was pooled or analyzed separately by groups.

Overall, multivariate methods (TPS/BE+VC, SAH+VC) performed better than their univariate counterparts on all samples. The results presented in this study are consistent with Stoyanova et al. (2017), who demonstrated a significant improvement in age-at-death estimation when the SAH score, TPS/BE, and VC methods were combined in a multivariate method. When examining the univariate regression models against the chronological age of the sample, the SAH score outperformed the TPS/BE and the VC methods. Results in this study consistently demonstrated that the magnitude and directions of error for the TPS/BE and VC methods were the highest among all methods. These results are consistent with Stoyanova's results on White American males, which demonstrated that the SAH score usually outperforms the TPS/BE method, and the VC method alone does not outperform either of the previous computational methods.

When comparing all computational methods for both groups, all five methods performed better on the Puerto Rican sample. Inaccuracy values were the lowest, ranging from 12.04 years for the SAH+ VC method to 14.91 years for the VC method, in comparison to the Mexican sample which presented inaccuracy levels ranging from 17.09 years to 20.57 years. Correlations between chronological age and estimated age were significantly higher for the Puerto Rican sample, ranging from .28 to .57. When the sample was pooled, correlations remained below .48, and when the Mexican sample was examined separately, all correlations for the computational methods were non-significant.

It would be tempting to argue that population differences are driving the relative better performance of the Puerto Rican sample against the Mexican sample. However, several issues with the age distribution of the sample might provide a better explanation as to why the Puerto Rican sample is performing better. First, prior publications have demonstrated that all five computational methods perform better on younger samples, thus attributing the low level of inaccuracies to the Puerto Rican sample (Slice and Algee-Hewitt 2015; Stoyanova et al. 2016; Stoyanova et al. 2017; Koterova et al. 2018). All five computational methods were developed on a sample of 93 White American males and half of those individuals were between the ages of 40 to 90 years of age, thus implying that the reference sample is slightly skewed towards younger individuals (16-39 years, n=48; 40-90 years, n=45). As previously mentioned, the Puerto Rican sample distribution is skewed towards younger individuals, while the Mexican sample is composed of older individuals. Furthermore, the Mexican sample is quite small in comparison to the Puerto Rican sample, so this might be the reason why no significant improvements are seen when the sample is pooled. Second, even though computational methods are being used to characterize the shape and morphology of the pubic symphysis, the age of each individual is calculated using a linear regression model, similar to the one used in the Suchey Brooks method. Thus, the same issues of producing age estimates for an unknown that are biased on the direction of the composition of the ages of the reference sample are going to be encountered.

When the magnitude and directionality of error were compared against prior studies analyzing the same computational methods (Stoyanova et al. 2016), overall, values are not too different (Table 7-1). Inaccuracy values presented for the original study (Stoyanova et al. 2016; Stoyanova et al. 2017) range from 10.79 years to 12.86 years, while inaccuracy values for the pooled Mexican and Puerto Rican sample range from 12.59 to 15.52 years. Bias values for both samples combined range from -0.02 to 7.70 on all five computational samples, while Stoyanova et. al. (2016) reported ranges between -1.82 to -2.73 years for their entire dataset. While the inaccuracy and bias levels are higher than the original reporting, it is worth noting that the original computational methods and linear regression models were developed using White American males. These are the same models used to calculate the estimated ages for the Latin American samples. Even though different trends were observed in the values of inaccuracies for both populations on all methods, inaccuracy values for all methods show that the average estimation error increased with the progression of age. Thus, it was demonstrated that as age increases, all methods tend to present more error.

A different study using 96 male pubic symphyses from four European collections provided bias ranging from -8.43 (VC method) to -15.67 (TPS/BE+VC (Kotevora et al. 2018). Inaccuracy values for the aforementioned study ranged from 14.15 to 16.96, which were much higher than when compared to the current study and the Stoyanova study. It is interesting to note

Table 7-1. Comparison of bias and inaccuracy values for the Kotevora et al. 2018, Stoyanova et al. 2017, and the current study.

Method	Kotevora et al. 2018		Stoyanova et al. 2017		This study	
	Bias	Inaccuracy	Bias	Inaccuracy	Bias	Inaccuracy
SAH Score	-13.58	15.70	-1.96	10.81	-2.53	13.87
TPS/BE	-15.54	16.75	-2.51	12.58	-10.86	15.58
VC	-8.43	14.15	-2.73	12.86	-2.95	15.47
TPS/BE + VC	-15.67	16.96	-2.21	11.39	-9.08	14.46
SAH+VC	-13.67	15.99	-1.82	10.79	-2.71	13.66

that the study using European males suggested that the multivariate analysis using the SAH+VC method and TPS/BE+VC method has the highest magnitude and directionality of error. Similar to previous studies, inaccuracy values for all methods showed that the average estimation error increased with the progression of age.

Some explanations for the higher error values on the Kotevora et al. (2018) study include the mean age distribution of the sample and different post-processing steps after laser scanning, like cleaning and trimming the pubic symphysis. First, as previously mentioned, all computational methods perform better on younger individuals. The sample used in the Kotevora et al. (2018) study is slightly skewed towards older individuals, with more than half of the sample between the ages of 41 to 83 years of age, with a mean age of 56.90 years. Kotevora and colleagues acknowledge that the higher error in directionality and magnitude is due to the composition of the sample.

A second potential source of error could be the use of a different scanner and postprocessing steps. Kotevora et al. (2018) used a less different structured light scanner (HP 3D Structure Light Scanner Pro S2), that utilizes projected light patterns with a camera system that captures the three-dimensional shape of the pubic symphysis. In contrast to the NextEngine 3D desktop scanner, the structured light scanner is known for providing a higher resolution with less scanning time. Previous publications have tested the structured light scanner's accuracy and reliability in human and non-human bones when examining sharp force and blunt force trauma (Gonzalez et al. 2015; Edwards and Rogers 2017), but it is unknown whether the output of these two scanners can be a source of error. However, when using the computational methods, the symphyseal phase needs to be manually selected from the rest of the bone, and the cleaning process depends to some extent on the resolution of the scan data. Thus, it is safe to assume that the better the resolution provided by the scanner, the less margin of error should be encountered when manually selecting the symphyseal phase. However, after laser scanning the pubic symphysis, the symphyseal face needs to be manually trimmed by the practitioner. Kim and colleagues (2017) have shown that even though the sources of error are minimal when comparing results from four practitioners with different levels of experiences, several potential sources of error are still present, like the lack of delimitation of the extremities on younger individuals when trimming the pubic symphysis, the presence or absence of a tubercle, and deciding which side is the ventral or dorsal side when loading the file on the *forAge* software. These are potential sources of error if the symphyseal face is not properly trimmed.

The first hypothesis of this study stated that that the Suchey-Brooks method, the Slice-Algee-Hewitt (SAH) Score method, the Bending Energy Method, and the Ventral Curvature method of the pubic symphysis will yield age estimates with higher error rates for samples of Mexican and Puerto Rican background than when using samples of European and African ancestry, as the methods were originally developed on non-Hispanic samples. Overall, based on the results obtained when examining the performance of all aging methods, it is safe to assume that the first hypothesis presented in this study can be accepted. It was demonstrated that, even though traditional and computational methods performed reasonably well on all samples, the direction and magnitude of error was significantly high on several age groups. Caution should be taken when attempting to age older individuals when using both traditional and computational methods, as the values of error are significantly higher. Although this examination was able to help quantify the magnitude of error between all techniques and provide information as to how appropriate it is to use each method on several Hispanic samples, samples from other Latin American regions and further analysis may help to better assess the performance of the methods, and the potential effects of secular change and interpopulation variability in Latin American samples. This will further help in determining whether the findings of this study may be extrapolated to model the collective population of Latin America or are limited to groups from Mexico and Puerto Rico.

Are population-specific methods needed for samples originating in Latin America?

Hypothesis two is based on findings from population genetic studies and morphological variation in skeletal remains, which suggest that Hispanic groups could be sufficiently different (e.g., genetically and morphologically), to the extent of being distinguishable between groups (Algee-Hewitt et al. 2018; Algee-Hewitt et al. 2017; Gonzalez Buchard et al. 2005; Moreno-Estrada et al. 2013,2014; Via et al. 2011). . Thus, the second hypothesis proposes that age estimation models developed for Hispanic samples will perform better if they are population specific. This hypothesis addresses whether or not Mexican and Puerto Rican samples can be separated as two different groups, and in turn, require two different age-at-death techniques, or if they should be pooled together in order to develop a single age-at-death technique.

First, in order to determine if significant differences are evident between estimated mean ages and chronological ages, a mixed linear model was performed. Results from this study demonstrated that no significant differences between age estimates and chronological ages are evident between the Mexican and Puerto Rican sample. When a third reference sample from Spain was included, no further differences were observed. After discovering that no significant differences are observed between groups, new univariate regression models were created with all three samples pooled. Surprisingly, when new univariate models were created using the aforementioned samples, no significant improvements in age-at-death estimation were reported.

When new univariate regression models were created using the Puerto Rican and Spanish samples alone, error rates were comparable to the original computational models created with White American males in the Stoyanova et al. (2017) study. In the aforementioned study, Stoyanova and colleagues report results for the SAH score with an R^2 value of 0.478 and a RMSE value of 14.15 years. For the current study, when new regression equations were created with the Puerto Rican, Mexican, and Spanish samples, the R^2 value decreased significantly (0.03); however, the RMSE value was comparable to the original study at 15.25 years. Similar results were obtained for the TPS/Bending Energy method and the Ventral Curvature method, where the R^2 was significantly lower, but the RMSE was close to the original values of Stoyanova et al. (2017).

It is worth nothing that when the Mexican sample was removed from the new regression models, the RMSE values decreased significantly. The new RMSE outperform the regression models of Stoyanova by 2.36 years for the TPS/Bending Energy method, 0.67 years for the SAH score method, and 1.93 years for the Ventral Curvature method. All of the new regression models were significant at a p=0.05; however, the R^2 decreased significantly. The new models were then subjected to cross validation using the Mexican sample as the unknown. Bias and inaccuracy values were calculated for all three computational methods and overall, no significant improvement was seen for the inaccuracy values, with most values ranging between 18 to 20 years. However, bias values improved significantly. Bias values decreased from values as high as

-16 to -6.09. Trends regarding the increase of bias and inaccuracies with increasing age were still a pressing issue with these new models.

Thus, it is reasonable to reject the hypothesis that age estimate models for samples originating in Latin American will perform better if they are population specific. Even though new regression models provide significant improvement in the RMSE, the directionality and magnitude of error provided when tested on the Mexican sample was not that much different than when using the original equations created with 96 White American samples. It is possible to assume that the improvement in the RMSE is mainly due to sample size rather than interpopulation variability. Stoyanova et al. (2017) demonstrated that by the addition of 40 samples, there is a substantial improvement in the R^2 and RMSE to the SAH score, TPS/BE, and VC method. It is worth noting that the original computational methods are based on 93 individuals, while the new regression models created with the Spanish and Puerto Rican samples are over double the sample size (n=234). This might be a potential factor for the improvement in the R^2 and RMSE. In addition, it should be noted that in the current study it was not possible to create new multivariate equations, like previously presented in the Stoyanova et al. 2017 study. As previously mentioned in the results section, the multivariate methods performed the best on all groups in this study. Thus, it is possible that the magnitude and directionality of error would have decreased for both samples if these new multivariate equations were developed. It is of great interest to create these new multivariate equations and examine the performance of these models on these samples in order to determine if the findings of this study are accurate.

It is important to highlight that the standards proposed in this study were derived using skeletal samples that originated from Mexico, Puerto Rico, and Spain and may not represent the entire aging variability between other groups in Latin America. This study presented theoretical evidence demonstrating that populations from Latin America could be sufficiently different, genetically and morphologically, to the extent of being distinguishable between groups. However, this study has demonstrated that no significant differences in estimated mean ages between Mexican and Puerto Ricans samples were detected when examining the mixed linear models and improvements with new linear regression equations. Rather than examining population genetic differences between groups, it is more pertinent to examine what factors are affecting growth and development, and how similar these factors are for both samples. Previous studies have suggested that socioeconomic status is a more influential factor affecting maturation differences than group affiliation (Schmeling et al. 2006; Schmeling et al. 2000; Langley-Shirley and Jantz 2010). It has been demonstrated that the critical factor that adds to the differences in ossification rates is the socioeconomic development of a given population (Schmeling et al. 2006; Schmeling et al. 2000). Although the genetic composition of an individuals might have a potential contribution to skeletal maturation, it is unknown if this potential is dependent on group differences.

Examining the population history of both countries can provide potential explanations for the lack of differences. First, during the first part of the 20th century both countries, Mexico and Puerto Rico, experienced profound economic and political transformations that affected the living standards of both groups. For example, Mexico underwent several events, such as the dictatorship of Porfirio Díaz and the Mexican Revolution, during the 19th century that affected the well-being of the population well past the 20th century. Nearing the end of the 19th century, the Porfirio Díaz administration took control over the economic and political opportunities of the country, promoting economic growth, modernization and political stability for the country, but at the expense of the rural and lower-class groups. Previous studies have shown that *peones* (e.g., hacienda workers) experienced slave like treatments by the *hacendados* (e.g. haciendas' owners), had limited amounts of food, long work hours and wages ranging from a high of .30 cents a day to a low of .23 cents a day (Katz 1974; Mora Torres 2011). Many considered the *peones* of several regions of Mexico as experiencing similar conditions to slavery in the Caribbean (Katz 1974).

Due to the negative effects of the Diaz administration in the lower and working class of the population, the Mexican Revolution took place during the beginnings of the 20th century (1910-1920). The revolution was considered to be one of the bloodiest wars that Mexico encountered, with over a million Mexican deaths during this time period. During the Mexican Revolution the population growth declined, and mortality rates increased. It is believed that over 5% (more than 1 million) of the entire Mexican population perished during this war.

In comparison with other Latin American countries, Mexico presents one of the highest percentages of illiteracy, especially among females (~15%) and about 11% of children present somatic growth stunting, with 47% of the children below the 15th percentile in US standards (Vazquez-Vazquez et al. 2013). While many countries throughout the world presented an increase in stature during the 19th and 20th century, Mexico experienced a decrease in the biological well-being of its population. Due to the economic hardship many Mexicans encountered during the 19th and 20th centuries, migration to the United States was a feasible solution to poverty. Feliciano (2000) estimates that outward migration to the United States increased from .2% to 2.2% of the population by the 1900's; however, she states that this migratory shift did not contribute significantly to the population decline of the century. However, Mora-Torres (2011) states that outward migration to the United States had in fact a big impact on the slow population growth in the 20th century. He states that migration started at 400,000 individuals by 1940 and three years later increased to 640,000. Mora-Torres believes that the growing cultural economy in the US and discovery of gold promoted a systematic outward flux of people to the United States.

In a similar way, Puerto Rico underwent similar transformations, including the end of the Spanish American war and an increase of migration to the US. By mid-19th century, Spain lost control of the island to the United States during the Spanish American War. Much of the agrarian economy was lost after years under the control of the United States, and by the end of the 19th century most of the sugar economy was decimated. In addition, the coffee and tobacco economy stagnated, and by the beginning of the 20th century both economies were reduced to only a few haciendas on the island. After the invasion of the United States, most of the island transitioned from a rural economy to a manufacturing and services economy. Previous studies have suggested that an increase in socioeconomic development occurred during the 1940's with a rise in employment, and a decrease in crime. Income per capital was higher than any other Latin American country and the standards of living were considered to be impressive. However, in comparison to the United States, disposable income during the 1990's was around $14,000 (U.S. currency) while on the island it was only $6,000 (U.S. currency). Even though the island was in good standing, it remained more similar to a developing country in Latin America than to the United States (Rivera-Batiz and Santiago 1996).

Between the 1970's and 1990, the island experienced a rise in unemployment, crime and substance abuse, high poverty rate and socioeconomic inequality. Due to the increase in unemployment, the island experienced an increase in emigration with more than 110,000 individuals departing between the 1970's and 1990's (Rivera-Batiz and Santiago 1996). Socioeconomic inequality became more apparent, with the lower socioeconomic class of the island receiving only 7% of the net income of the island. Social inequalities were more prominent in women, where more than 60% were below the poverty line and 54% of males fell below the poverty line. An increase in deaths was mainly due to an AIDS epidemic, while secondary factors included car accidents and homicides. A decrease in birth rates was seen from 1980's to 1990's due to more females attaining a college education and opting to have fewer children.

Based on the historical effects that took place in both countries, it is feasible to assume that these two groups probably experienced similar economic and political transformations that affected the living standards of each group. Even though we can reject the hypothesis that age estimate models for these two groups will perform better if they are population specific, we must proceed with caution when examining other groups not included in this study, as they might present significant difference in secular change or interpopulation differences.

Another significant point that should be highlighted in this study is the use of computational methods to estimate age-at-death. This study shows that current computational methods and 3D laser scans are a reliable option to estimate age-at-death on samples originating from Mexico and Puerto Rico. The results presented here demonstrate that by increasing the sample size of the reference sample on the original computational regression equations, it might be possible to increase the R^2 and decrease the RMSE by more than 2 years. It could be argued that by providing such low RMSE levels, computational methods could outperform the Suchey-Brooks method. Furthermore, computational methods eliminate the subjectivity issues that come along when scoring the features of the pubic symphysis, making the process of aging the skeletal element a less ambiguous task. During the data collection process, the laser scanner was easy to transport from collections to collection and data collection only took several minutes per individual. The cleaning and postprocessing steps were easy to understand and perform. Overall, it has been shown that computational methods offer an improvement in age-at-death estimation for some age cohorts, but most importantly, improvements in objectivity when estimating age-at-death from the pubic symphysis.

Recommendations for future forensic cases when estimating age-at-death in samples originating from Latin America

When attempting to estimate age-at-death on potential samples originating from Mexico and Puerto Rico, it is recommended to use the univariate computational regression equations with the Spanish and Puerto Rican samples. This recommendation is suggested due to two main findings; the low RMSE produced by each equation and the similar magnitude and directionality of error produced when testing the equations with a sample originating from Mexico. As previously mentioned, one of the main concerns with these new computational equations is the age point estimate presented, instead of an age range with a confidence interval. In order to solve this issue, this study suggests the use of the RMSE as part of the error rate when reporting an age estimate in a forensic context. The RMSE is a measure of error around the regression line, similar to the standard deviation, which is a measure of variability within the mean. Thus, by using the 68-95-99.7 rule in a normal distribution, the probability of a random data point landing within 1 (68%), 2 (95%), or 3 (99.7) standard deviations of the mean can be translated by using the RMSE. Thus, at a confidence interval of 68%,

$$.68 \approx probability\ (\mu - RMSE \leq age\ estimated\ \leq \mu + RMSE)$$

Where μ is the mean of the distribution and the $RMSE$ is the root mean square error associated with the linear regression equation used for age-at-death estimation. When attempting to establish the probability of a random data point (estimated age) landing within 2 standard deviations, or in this case, RMSE, from the mean, the following equation should be used;

$$.95 \approx probability\ (\mu - RMSE(x2) \leq age\ estimated\ \leq \mu + RMSE(x2)$$

Limitations

Several limitations were encountered during this study. First, the sample size for both populations is not ideal. As previously mentioned, the sample composition does not represent all age groups. As a result, all aging methods could not be tested on older individuals for the Puerto Rican sample and younger individuals on the Mexican sample. Ideally, a bigger sample size with a uniform distribution should be used to improve the results of this study. A significant issue encountered during data collection included the lack of preservation of the pubis and issues while attempting to scan samples with discoloration or cartilage still present. It is worth noting that all samples used in this analysis originated at the Medical Examiner's Office from each country, and many of the individuals included in each collection had to be macerated. Thus, sometimes the cartilage in between each pubic symphysis was not removed. This issue was frequently encountered on all skeletal collections and many individuals had to be excluded from the study.

Other issues encountered include extended periods of water maceration, thus the porosity and composition of the pubic symphysis was compromised.

Second, the sample size was also considerably reduced when the female sample was removed. A small sample size of females and a lack of computational linear regression equations for females in the SAH score, the BE, and the VC score were the main reasons for removing the females from this study. However, it is imperative to obtain more female samples in order to create appropriate linear regression equations for females.

Third, demographic information, like socioeconomic status, place of birth, and occupation were not accessible at the time of data collection. Obtaining this information is crucial to make inferences about population differences and how it might have affected interpretation regarding population specificity. Future directions for the current study include an increase in the sample size and inclusion of females, and demographic data for each individual in order to better analyze possible aging differences between populations. In addition, a recommendation to include the current samples (Mexican, Puerto Rican and Spanish samples) into the original sample size of 94 White males will be presented to further examine if population specificity is needed.

CHAPTER EIGHT
CONCLUSION

This study attempted to quantify the magnitude and directionality of error of several aging techniques utilizing the pubic symphysis for two groups in Latin America: Mexicans and Puerto Ricans. This study also examined the potential need for population specificity in aging techniques for these two reference samples. The results of this study indicate that both traditional and computational methods perform reasonably well on middle-aged (35-50) individuals. When comparing methods, the computational methods perform better on younger and older individuals than when using the Suchey-Brooks method (i.e., traditional). It was also shown that new population specific regression equations for computational methods do not necessarily improve age estimates. Several important points from this study are described below:

1. Caution should be taken when using the Suchey-Brooks method on younger or older individuals of Latin American origin. The method tended to overestimate age on individuals under 35 years of age and underestimate age on individuals above 50 years of age. These results are similar to previously published studies applying the Suchey-Brooks method to other populations across the world.
2. In comparison to the Suchey-Brooks method, the computational methods also performed better on younger individuals. Computational methods performed better than the Suchey-Brooks method on older individuals, but overall, the magnitude and directionality of error was still significantly high. When all computational methods were compared against each other, the multivariate methods performed better on all samples. Similar to previously

published studies examining age-at-death techniques, the magnitude and directionality of error increased as chronological age increases.

3. When new population specific linear regression equations were created for the SAH score, TPS/BE, and VC method, no significant improvements in age estimates were reported. However, a significant improvement in the RMSE was seen for all equations. It is suggested that this improvement is due to sample size rather than population variability. It is highly recommended to include other samples from different geographical locations in order to examine the full potential of these equations when estimating age-at-death.
4. No interpopulation differences in age estimates were observed between the Mexican and Puerto Rican sample when all computational methods were used. Potential explanations for the lack of differences can be due to similar factors (i.e., socioeconomic status, access to health services, and living standards) affecting both groups. A short summary of the population histories of both countries during the end of the 19^{th} and 20^{th} century demonstrated that both countries experienced profound economic and political transformations that affected the living standards of the population.
5. This study has shown that computational methods utilizing 3D laser scans of the pubic symphysis could ultimately outperform traditional methods (i.e., bone morphology matched to a phase), and provide improvement in objectivity when aging an individual. Further development of this technique has the potential to provide age estimates with less error while bypassing the known subjectivity from the practitioner.

As stated in many previous publications, caution should be applied in the use of traditional aging techniques on individuals over 50 years of age. We can also add that computational methods should also be used with caution when aging older individuals. The results from this study have demonstrated once again that age estimation is not an exact science due to the highly variable nature of human senescence. Age-at-death estimation is only an approximation of chronological age using an estimate that is derived from biologically imperfect features that are influenced by many internal and external factors that we cannot control.

Results from this study have demonstrated that no perfect method is available to estimate age-at-death for populations from Latin America. Until better methods and more thorough research studies are performed on groups originating from Latin America, it is recommended to use the revised univariate regression models presented in this study while using 3D laser scans of the pubis symphysis. The computational methods presented in the current study present an easy to use scanning technique, and a friendly user interface, allowing the practitioner to bypass the known subjectivity of current traditional methods. It is hoped that other populations from Latin America are included in further research in order to better represent the variation in age related changes seen within and between individuals for the groups.

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