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STATURE ESTIMATION IN SOUTH AFRICAN JUVENILES AND ADULT FEMALES

Desiré M. Brits

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2016

DECLARATION

I, Desiré M. Brits, declare that this thesis is my own work. It is being submitted for the Degree of Doctor of Philosophy (PhD) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination, at this or any other University.

_____

Desiré M. Brits

_____day of_____20_____in_____

Presentations arising from this thesis:

- Brits, D., Bidmos, M.A., Manger, P.R. (2013) Assessing the accuracy of Fully's soft tissue correction factor for stature estimation in black South African females. Oral presentation at the 41st Annual Conference of the Anatomical Society of Southern Africa.

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- Brits, D., Bidmos, M.A., Manger, P.R. The accuracy of the anatomical method for stature estimation in Black South African females. In submission.

- Pillay, S., Brits, D., Essan, T. The accuracy and repeatability of skeletal measurements collected from Magnetic Resonance Imaging Scans. In preparation.

ABSTRACT

Research on stature estimation in sub-adult is often hampered by the general lack of modern skeletal collections with contextual data. To overcome this limitation the current study utilised magnetic resonance imaging (MRI) scans of living participants to assess stature estimation methods.

Firstly, the precision of osteometric data collected from MRI scans was evaluated by comparing measurements collected from MRI scans of 36 fleshed cadaver limbs to similar measurements collected from the same dry bones. Results showed no significant differences between MRI and dry bone measurements, except for the epicondylar breadth of the femur, and as such MRI can be used to accurately collect osteometric data.

Studies have questioned the accuracy of the anatomical method, as this method continuously underestimates stature. This has been related to the use of a universal soft tissue correction which some suggest are sex- and population-specific. Total skeletal height was calculated from measurements collected from MRI scans of 30 Black South African adult females and living stature was estimated using the methods proposed by Fully, Raxter *et al*. and Bidmos & Manger. Results indicated that the anatomical method as described by Fully and Raxter et al. significantly underestimated stature while the method proposed by Bidmos & Manger significantly overestimated stature. A new soft tissue correction factor specific for Black South African females was calculated which improved the accuracy of stature estimates.

Sub-adult skeletal remains are mainly evaluated for age estimation, but regrettably little information is available regarding the estimation of sub-adult stature. Various measurements of the femur and tibia as well as the total skeletal height were attained from MRI scans of 59 Black

South African sub-adult males and females and used to describe sub-adult stature. Results indicated very strong statistically significant positive correlations between the measurements and living stature, with stature estimation regression equations characterized by small standard error of estimates, comparable to that reported for adults. Descriptions of stature are thus encouraged as it can add valuable information to the biological profile of sub-adult remains. This study is relevant to forensic applications where it is necessary to quantify stature from skeletal remains.

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CHAPTER ONE

Introduction and Chapter Outline

1.1. INTRODUCTION

Forensic anthropologists have seen a significant increase in case workloads where they are relied upon to assist with the compilation of biological profiles (Wilson et al., 2010). According to Statistics South Africa a total of 453 360 deaths were documented in South Africa in 2014. Of these deaths 47 761 were recorded as non-natural. Non-natural deaths are deaths related to non-natural causes such as violence and accidents and based on the Inquest Act of South Africa (No. 58 of 1959) they are subject to an autopsy and legal investigation (Statistics South Africa, 2015). These bodies are presented to forensic pathologists for post-mortem examination and in cases where the remains are found severely decomposed or skeletonized, the help of a forensic anthropologist is sought (Barrier & L'Abbé, 2008). Forensic anthropologists assist by providing biological information of the deceased, such as age-at-death, ancestry, sex and stature along with other individualizing factors that might be useful in establishing a presumptive identification (İşcan & Steyn, 2013). Of importance to this study is the estimation of stature, which refers to the "natural standing height of an individual" (Baines et al., 2011, p. 95). Stature is often classified as a factor of individualization as it describes a feature which is unique to an individual (İşcan & Steyn, 2013). Stature can thus contribute to the positive identification or exclusion of an unknown individual (İşcan & Steyn, 2013; Moore & Ross, 2013).

Two avenues are available for the estimation of stature; i.e. the anatomical method and the mathematical method, with an abundance of literature describing these methods. Studies have, however, questioned the accuracy of the anatomical method as it significantly underestimates stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Bidmos & Manger, 2012), especially in Black South African males (Bidmos, 2005; Bidmos & Manger, 2012). These inaccuracies also call

into question the validity of the mathematical method as various regression equations are based on stature estimates calculated using the anatomical method.

Stature is routinely assessed during the skeletal analysis of adult remains, but is rarely attempted when dealing with sub-adult skeletal remains (Lewis & Rutty, 2003; Smith, 2007; Cardoso, 2009; Sutphin & Ross, 2011). This is related to the general lack of sub-adult skeletal collections with known demographic information, available for research (Sundick, 1977; Lewis & Rutty, 2003). A few sub-adult stature estimation regression equations, derived from radiographs are available, but the accuracy of these equations has been questioned, as the geometric magnification introduced by X-rays are seldom accounted for (Feldesman, 1992; İşcan, 2005; Smith, 2007). Additionally, no standards are available to reconstruct stature from the skeletal remains of South African sub-adults.

1.1.1. Study aims and objectives

The overall aims of this thesis are to: (1) assess the accuracy of the anatomical method for stature estimation in Black South African adult females; and (2) describe stature estimation in Black South African sub-adults using Magnetic Resonance Imaging (MRI) scans.

The study has the following objectives:

1. Assess the accuracy and reliability of skeletal measurements collected from MRI scans.
2. Evaluate the accuracy of the soft tissue correction factors proposed by Fully (1956) and Raxter et al. (2006) for the estimation of stature in Black South African adult females.
3. Calculate a new soft tissue correction factor for the estimation of living stature in Black South African adult females.

4. Determine the relationship between living stature and lower limb long bone lengths in Black South African sub-adult males and females
5. Determine the relationship between living stature and total skeletal height in Black South African sub-adult males and females
6. Derive regression equations for the estimation of sub-adult stature from lower limb long bones and total skeletal height in Black South Africans

1.2. CHAPTER OUTLINE

1.2.1. Chapter two: Literature review

This chapter provides a review of the literature available on stature estimation for adult and sub-adults as well as the rationale and motivation for the thesis.

1.2.2. Chapter three: The accuracy and repeatability of skeletal measurements collected from magnetic resonance imaging scans

Some skeletal collections are often no longer representative of the population from which they have been derived (Hunt & Albanese, 2005; L'Abbé et al., 2005; Komar & Grivas, 2008; Dayal et al., 2009). This, along with the general lack of sub-adult skeletal collections with known demographic details, has led to numerous skeletal publications utilising various image modalities of the living, but questions regarding the accuracy of such skeletal measurements have arisen. Therefore, the aim of the current study was to assess the accuracy and repeatability of skeletal measurements collected from Magnetic Resonance Imaging (MRI) scanograms. MRI scans were collected from 36 cadaveric lower limbs from which standard anthropometric measurements were taken, using the image software package OsiriX. Following the MRI scans all soft tissue was

manually removed and the measurements repeated, using calipers and an osteometric board. Finally the remains were macerated and the same measurements were collected from the dry bones. Differences between the measurements were assessed with paired and one sample t-tests, as well as the Bland-Altman method. Results indicated statistically significant differences between the dry and wet bone measurements; however, no differences were found between dry bone and MRI measurements, except for the epicondylar breadth of the femur. Furthermore, the Bland-Altman method showed no bias in the differences between the measurements, except for the ankle height measurements. The mean differences between MRI and dry bone measurements were less than 2 mm, which is within the range of error accepted by forensic anthropologists and as such MRI scans can be used in the collection of accurate osteometric data.

1.2.3. Chapter four: The accuracy of the anatomical method for stature estimation in Black South African females

The anatomical method has repeatedly been reported as the most accurate method for stature estimation (Lundy, 1985, 1988; Ousley, 1995; Bidmos, 2005; Maijanen, 2009), but investigation has shown that it continuously underestimates stature. This underestimation is believed to be related to the use of universal soft tissue correction factors. Therefore, the aim of this study was to assess the accuracy of the soft tissue correction factors in a living population of Black South African females and to subsequently calculate a new soft tissue correction factor, specific for stature estimation in this population group. Thirty Black South African adult females voluntarily participated in this study and underwent a full body Magnetic Resonance Imaging (MRI) scan. Living stature was measured with a stadiometer and total skeletal height (TSH) was calculated from the MRI measurements. Stature was estimated from the TSH of each participant

using Fully's, Raxter et al. and Bidmos & Manger's methods. Results indicated strong, statistically significant positive correlations between living and estimated statures, however, paired t-tests revealed that living stature was significantly underestimated using Fully's and Raxter et al.'s methods, while the method by Bidmos & Manger significantly overestimated stature. A lack of statistically significant correlations between soft tissue correction factors and the total skeletal height was found. Likewise, an absence of statistically significant correlations between age and the estimation error, with and without age adjustments were also observed. Subsequently, a new soft tissue correction factor, specific for stature estimation in Black South African females was calculated. The newly proposed regression equation presented improved stature estimation accuracies for this population group.

1.2.4. Chapter five: Stature estimation from the femur and tibia in Black South African sub-adults

Stature estimation can play a role in the positive identification of unknown individuals and as such it is routinely assessed during the examination of adult remains. Unfortunately, this is not a standard procedure when dealing with sub-adult remains due to the general lack of standard procedures for the estimation of sub-adult stature. The aim of this study was therefore to derive regression equations for the estimation of stature in black South African sub-adults. Fifty nine black South African sub-adult males and females, aged 10-17 years, voluntarily participated in the study by undergoing a full body Magnetic Resonance Imaging (MRI) scan. Living stature was measured with a stadiometer and the maximum and diaphyseal lengths of the femur and tibia were measured from the MRI scans using the image processing software OsiriX. Pearson's correlation coefficients and linear least square regression analyses were used to assess the correlations

between living stature and the measurements and to generate sub-adult stature estimation equations for males, females and a combined sex sample. Measurements of the femur, tibia and the combined measures thereof showed strong statistically significant positive correlations with living stature, while the obtained regression equations were characterized by low standard error of estimates. The strong correlations and low standard error of estimates are comparable to stature estimation models reported for Black South African adults and therefore these variables can be considered good estimators of sub-adult stature which will contribute valuable information to the biological profile of unidentified sub-adult skeletal remains.

1.2.5. Chapter six: Assessing the use of the anatomical method for the estimation of sub-adult stature in Black South Africans

Stature estimation is rarely attempted in sub-adults due to the general lack of available standards as a result of the dearth of sufficiently large sub-adult skeletal collections with known demographic information. To overcome this problem sub-adult research mainly relies on modern imaging modalities. In the current study Magnetic Resonance Imaging (MRI) scans were used to assess the use of the anatomical method for stature estimation in sub-adults. A total of 53 Black South African sub-adult males (n=24) and females (n=29) aged between 10 and 17 years participated in the study by voluntarily completing a full-body MRI scan. Living stature was measured with a stadiometer and the skeletal elements that contribute directly to stature were measured from the MRI scans and summed to compute the total skeletal height. Total skeletal height was computed from the diaphyseal, maximum and physiological long bone lengths and correlated to living stature using Pearson's correlations. Subsequently least squares regression equations were generated for the estimation of sub-adult stature. Results indicated strong,

statistically significant positive correlations between living stature and total skeletal heights in sub-adult males, females and a combined sex sample. The regression equations were characterized by small standard error of estimates which are comparable to that reported for Black South African adults. Based on these results the anatomical method can be used to accurately describe living stature in Black South African sub-adults. This method is therefore encouraged as it will add valuable information when dealing with unknown sub-adult skeletal remains.

1.2.6. Chapter seven: Discussion and conclusion

The final chapter provides a summary of the results of the studies that form this thesis, along with implications for stature estimation in Black South African adults and sub-adults. It also highlights the limitations of the current study and provides suggestions for future research.

CHAPTER TWO

Literature Review

2.1. INTRODUCTION

Traditionally, when dealing with skeletal remains, effort is put towards describing the age, sex, ancestry and stature represented by the remains (Dirkmaat et al., 2008; İşcan & Steyn, 2013). Of these attributes, stature has been described as the most straightforward characteristic to assess (Komar, 2003; Cardoso et al., 2016).

2.2. ADULT STATURE ESTIMATION

Stature is routinely assessed when dealing with adult skeletal remains and numerous publications describing stature estimation exist. To date, two methods are available to estimate stature and include the mathematical and the anatomical methods (Lundy, 1985; Sjøvold, 2000; Moore & Ross, 2013).

2.2.1. Mathematical method

The mathematical method is the most commonly used stature estimation method and allows for the reconstruction of stature from a single bone or a combination of bones (Formicola, 1993, Tatarek et al., 2005; Moore & Ross, 2013). The mathematical method consists of two components: (1) stature:bone ratios; and (2) regression analyses (Moore & Ross, 2013).

2.2.1.1. Bone:stature ratio

Sue (1755), as cited by Stewart (1979), initially calculated bone:stature ratios in an attempt to study the change in body proportions based on the growth of various bones compared to stature; however, the first standardized formulae for the estimation of stature were only introduced in 1881 by Topinard (Stewart, 1979; Moore & Ross, 2013). Following suggestions by Topinard, Rollet

(1888) published the first stature estimation tables which related the length of various bones to stature. These tables were later re-organized by Manouvrier (1892) who excluded all the individuals older than 60 years of age and also recommended the conversion of cadaveric height to living stature (Stewart, 1979; Moore & Ross, 2013). Following this, Hrdlička in 1939 created bone:stature ratios by measuring the long bones of American dissection cadavers (Stewart, 1979; Krogman & İşcan, 1986; Moore & Ross, 2013). Of specific interest is the relationship between the femur and stature which has been calculated to be 3.74 (Sjøvold, 2000). This ratio is fairly consistent among sexes and population groups and was initially believed to be universal (Sjøvold, 2000); however, reports by Feldesman and Fountain (1996) have illustrated statistically significant differences in this ratio between various population groups.

The femur:stature ratio has been shown to produce accurate estimates of stature for individuals of average height, but tends to underestimate and overestimate stature for short and tall individuals, respectively (Sjøvold, 2000; Moore & Ross, 2013). Use of bone:stature ratios are currently overshadowed by the use of regression equations (Moore & Ross, 2013).

2.2.1.2. Regression analyses

The data published by Rollet (1888) was subjected to statistical analysis by Pearson (1899), who is responsible for the birth of one of the most commonly used methods today, regression analyses (Stewart, 1979; Krogman & İşcan, 1986; Moore & Ross, 2013). Regression analyses can be undertaken *inter alia* on comparisons between bone lengths and stature (Raxter et al., 2006; Giurazza et al., 2012; Moore & Ross, 2013). It is a quick and easy method which can be applied to individuals of known sex and population affinity (İşcan, 2005; Cardoso et al., 2016). Many articles have been published providing regression equations for the estimation of stature for males

and females from various population groups including, Chinese (Zhang et al., 2016), Filipino (Taduran et al., 2016), Ghanaian (Abledu et al., 2016), Indians (Jagadish Rao et al., 2009; Singh et al., 2011; Khangura et al., 2015), Italian (Giurazza et al., 2013), Japanese (Hasegawa et al., 2009; Hishmat, 2015), Nigerian (Ekezie et al., 2015), North American (Dupertuis & Hadden, 1951; Trotter & Gleser, 1952; Trotter & Gleser, 1958), Portugal (De Mendonça, 2000; Cordeiro et al., 2009), Saudi Arabian (AlQahtani, 2015), South-East Asian (Hossain et al., 2016), South African (Lundy & Feldesman, 1987; Bidmos, 2006; Dayal et al., 2008; Pininski & Brits, 2014) and Spanish (Muñoz et al., 2001; Rodríguez et al., 2015) populations.

Most regression equations have been derived from long limb bones, especially those of the lower limb, as these equations yield the most accurate stature estimations (Dupertuis & Hadden, 1951; Trotter & Gleser, 1958; Lundy & Feldesman, 1987; Dayal et al., 2008; Hasegawa et al., 2009; Hishmat, 2015). This is due to the fact that lower limb long bones contribute directly to stature and as such are highly correlated to stature (Raxter et al., 2006). A number of articles have also been published on the estimation of stature from other skeletal remains such as the skull (Chiba & Terazawa, 1998; Ryan & Bidmos, 2007; Giurazza et al., 2012), cranial sutures (Jagadish Rao et al., 2009), teeth (Khangura et al., 2015; Hossain et al., 2016), vertebrae (Klein et al., 2015; Rodríguez et al., 2015), sacrum (Karakas et al., 2010; Pininski & Brits, 2014), sternum (Singh et al., 2011; Marinho et al., 2012), scapula (Giurazza et al., 2013), clavicle (Rani et al., 2011; Balvir et al., 2012), calcaneus (Bidmos & Asala, 2005; Bidmos, 2006; Zhang et al., 2016) and metatarsals (De Groote & Humphrey, 2011). A few studies are also available to assist with the reconstruction of stature from fragmented remains (Steele & McKern, 1969; Simmons et al., 1990; Craig, 1995; Bidmos, 2008a, 2008b).

Stature estimation by means of regression equations is very accurate, although it has been shown that regression analyses tend to underestimate stature in tall and overestimate stature in short individuals (Sjøvold, 2000). Moreover, studies have also questioned the accuracies of some of these equations, especially in South Africa, as most of these equations have been derived from questionable documented cadaver lengths (Bidmos, 2005) or from stature estimates produced using Fully's (1956) method which has been shown to significantly underestimate stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Bidmos & Manger, 2012).

Unfortunately, the estimation of stature from skeletal remains is becoming increasingly difficult as some populations continue to grow taller (İşcan, 2005). This makes stature estimation methods, especially regression analysis, time-specific and as such they must be constantly re-evaluated (Trotter & Gleser, 1958; Ousley, 1995; İşcan, 2005; Cardoso et al., 2016).

2.2.2. Anatomical method

The anatomical method, also known as Fully's method or the complete skeleton method, was initially described by Dwight in 1894 (cited in Lundy, 1985) and later refined by Fully (1956). Dwight's (1894) method involved the articulation of the skeleton with clay, which represented cartilaginous structures between bones, and the subsequent linear measurement of the articulated skeleton (Lundy 1985).

Fully's method on the other hand was based on the estimation of skeletal height which consisted of the sum of individual bones that contribute directly to stature, i.e. basi-bregmatic height of the skull, height of the vertebral column (C2-S1), length of the femur and tibia and the articulated height of the talus and calcaneus. A soft tissue correction factor was also added to the calculated skeletal height to reconstruct living stature (Fully, 1956). The method described by

Fully (1956) was derived from the skeletal remains of European males and Stewart (1979) cautioned against the use of this method, pending the assessment of the accuracy of the method on taller individuals from different populations.

The anatomical method is accepted by many as the most accurate stature estimation method (Stewart, 1979; Lundy, 1985; Formicola, 1993; Ousley, 1995; Raxter et al., 2006; Baines et al., 2011), benefiting from the inclusion of all the skeletal elements that make up height, which inherently also includes variations associated with differing body proportions. This method further benefits from the use of a single soft tissue correction factor which was initially thought to be independent of sex and population affinity (Lundy, 1985; Ousley, 1995; Raxter et al., 2006).

The accuracy of Fully's method (1956) was initially assessed and confirmed by Lundy (1988); however, studies have questioned the accuracy and applicability thereof as it continuously underestimates stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Maijanen, 2009; Bidmos & Manger, 2012), especially amongst Black South Africans (Bidmos, 2005; Bidmos & Manger, 2012). This underestimation has been associated with the use of the universal soft tissue correction factor (King, 2004; Bidmos, 2005; Raxter et al., 2006) which has been suggested to be sex- (Bidmos, 2005) and population-specific (King, 2004; Bidmos, 2005). Poor measurement definitions and age have also been put forward as factors contributing to the stature underestimation provided by Fully's (1956) method (Raxter et al., 2006).

2.3. SUB-ADULT STATURE ESTIMATION

Stature is rarely estimated in sub-adults (Kondo et al., 2000; Lewis & Rutty, 2003; Smith, 2007; Cardoso, 2009; Sutphin & Ross, 2011) as it is believed that continual growth of children nullifies the validity of these estimates (Lewis & Rutty, 2003). However, Snow and Luke (1970),

as cited by Smith (2007), emphasised its importance when stature estimation proved crucial during the separation of skeletal remains from sub-adult females of similar ages. Literature on sub-adult stature estimation is limited and forms part of a general shortfall of sub-adult standards which is thought to be related to the insufficient numbers of sub-adult skeletal collections, together with the absence of known demographic information for these remains (Telkkä et al., 1962; Sundick, 1977; Stewart, 1979; Kondo et al., 2000; Lewis & Rutty, 2003; İşcan & Steyn, 2013). Notwithstanding these limitations, a few sub-adult stature estimation publications are available (Balthazard & Dervieux, 1921; Smith, 1939; Olivier & Pineau, 1958, 1960; Palkama et al., 1962; Telkkä et al., 1962; Virtama et al., 1962; Fazekas & Kósa, 1978; Feldesman, 1992; Visser, 1998; Kondo et al., 2000; Ruff, 2007; Smith, 2007; Grivas et al., 2008; Krishan et al., 2011; Krishan et al., 2012b).

2.3.1. Mathematical method

To date only the mathematical method has been described in the literature for stature estimation from sub-adult skeletal remains and includes bone:stature ratios and regression analyses.

2.3.1.1. Bone:stature ratio

Little information on sub-adult bone:stature ratios is available and includes the bone:stature ratios described above by Sue (1755), as cited by Stewart (1979), and work described by Feldesman (1992).

Feldesman (1992) explored the relationship between the femur and living stature in sub-adults aged between 8 and 18 years and its use for sub-adult stature estimation. It was found that this ratio differed significantly between sub-adults and adults. The author also noted a statistically

significant difference in the ratio between 8 to 11 and 12 to 18 year olds. Further analyses illustrated that this ratio varied significantly between sub-adult males and females, which was related to the differences associated with adolescent growth spurts. It was proposed that sex-specific femur:stature ratios be used, especially for adolescents (12-18 years), to produce reasonable estimates of stature (Feldesman, 1992).

2.3.1.2. Regression analyses

A few studies have computed regression equations for sub-adult stature estimation. These equations rely on the correlation between living stature and the diaphyseal lengths of long bones commonly measured on radiographs of living children (Palkama et al., 1962; Telkkä et al., 1962; Virtama et al., 1962; Fazekas & Kósa, 1978; Visser, 1998; Ruff, 2007; Smith, 2007). Diaphyseal long bone lengths in sub-adults studies are more commonly used to predict age (Krogman & İşcan, 1986; İşcan & Steyn, 2013; Stull et al., 2014a) or growth rates (Lewis & Rutty, 2003) and have only recently been utilised for stature estimation.

Current regression equations for sub-adult stature estimation mainly include regression equations derived from the upper and lower limb long bones (Palkama et al., 1962; Telkkä et al., 1962; Virtama et al., 1962; Fazekas & Kósa, 1978; Visser, 1998; Ruff, 2007; Smith, 2007); however, regression equations from fleshed body segments are also available and included stature estimation equations from the head (Vinitha et al., 2015), upper limb (Banik et al., 2012), forearm (Song-in et al., 2013), hand (Ibegbu et al., 2015), finger (Krishan et al., 2012a) and foot (Grivas et al., 2008; Krishan et al., 2011; Krishan et al., 2012b) measurements.

The sub-adult stature estimation regression equations are often sex-specific and have also illustrated age cohort specificity (Telkkä et al., 1962). Additionally, a number of authors have

found that these equations are more accurate and reliable when applied to the population from which they have been derived, due to growth and nutritional differences found between various countries and population groups (Telkkä et al., 1962; Smith, 2007; Cardoso, 2009). There are no regression equations available to reconstruct stature for South African sub-adults.

Recent studies have, however, questioned the accuracy of regression equations derived from radiographs due to the magnification introduced by X-rays, which some studies account for and others omit (Feldesman, 1992; Ruff, 2007; Smith, 2007; İşcan, 2005).

2.3.2. Anatomical method

Sub-adult stature estimation using the anatomical method is yet to be described. Kondo et al. (2000) reported on the estimation of stature of a Neanderthal sub-adult using a method similar to that described by Dwight (1894). The authors described reconstructing and re-articulating the well preserved skeletal remains before directly measuring stature. Further accommodations were made for the shrinkage associated with the cast as well as the curvature of the vertebral column and the distances between articulating bones. Subsequently a soft tissue correction factor, incorporating the thickness of the scalp and the sole of the feet, was added. The authors compared their anatomically measured stature to that derived from regression equations described by Telkkä et al. (1962). The regression equations produced varying estimates of stature with large ranges that were attributed to differences associated with changing body proportions. The anatomical method described by Kondo et al. (2000) included all the skeletal elements that contribute directly to height and as such eliminated errors related to differences found in body proportions. This is thought to make the anatomical method more accurate for the reconstruction of stature (Kondo et al., 2000).

2.4. STATURE ESTIMATION METHODOLOGY

To allow for the development of stature estimation methods, information regarding stature and bone measurements is needed (Sjøvold, 2000), and in South Africa researchers rely heavily on the data reported in various skeletal collections, such as the Raymond A. Dart Collection of Human Skeletons (Dayal et al., 2009) and the Pretoria Bone Collection (L'Abbé et al., 2005). Unfortunately, information relating to living stature is not available for individuals housed in these collections and cadaveric height is mostly reported (Bidmos, 2005; Dayal et al., 2009). Research has shown that cadaveric height is larger than living stature and as such stature estimation methods described from cadaveric height requires a correction factor to convert the stature estimates into living stature (De Mendonça, 2000; Cardoso et al., 2016). Unfortunately there is no agreement on the magnitude of this correction factor, if it is even necessary (Cardoso et al., 2016).

Estimates of stature are also affected by age. Stature significantly decreases with an increase in age (Trotter & Gleser, 1951) due to the loss of elasticity of soft tissues and the compression of the intervertebral discs and vertebral bodies (Trotter & Gleser 1951; Galloway, 1988; Cline et al., 1989; Sjøvold, 2000; Raxter et al., 2006; Niskanen et al., 2013). The proposed rate of this decline is 0.6 cm per decade (Trotter & Gleser, 1951; Sjøvold, 2000); however, it is unclear when this reduction commences with authors proposing 30 (Trotter & Gleser 195; Raxter et al., 20061), 40 (Cline et al., 1989) and 45 (Galloway, 1988) years of age. Cline et al. (1989) found that the decrease in stature between the ages of 20 and 60 years is very small and are comparable to the daily diurnal variation. It has also been proposed that the rate of decline is similar in all population groups (Trotter & Gleser; 1951), although research has shown that it is sex specific (Galloway, 1988; Cline et al., 1989).

Stature estimation research is further encumbered by the lack of or incorrect documentation of cadaveric heights (Lundy, 1983; Bidmos, 2005; Hunt & Albanese, 2005; L'Abbé et al., 2005; Komar & Grivas, 2008; Dayal et al., 2009) and researchers often rely on the anatomical method to estimate living stature. Studies have questioned the accuracy of the anatomical method as it continuously underestimates stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Bidmos & Manger, 2012). These inaccuracies have called into question the accuracy of stature estimation regression equations derived from stature estimates using the anatomical method and future efforts are needed to re-evaluate the applicability of these stature estimation equations.

Skeletal collections are often effected by sampling bias (Komar & Grivas, 2008) with many collections having an over representation of Black males and individuals representative of lower socio-economic standings such as the Robert J. Terry Anatomical Collection (Hunt & Albanese, 2005), The Pretoria Bone Collection (L'Abbé et al., 2005), Maxwell Museum Documented Collection (Komar & Grivas, 2008) and the Raymond A Dart Collection of Human Skeletons (Dayal et al., 2009). These collections are also often no longer representative of the population from which they have been derived due to secular change (Meadows & Jantz, 1995; Ousley & Jantz, 1997; Meadows Jantz & Jantz, 1999; Dirkmaat et al., 2008; Komar & Grivas, 2008). Secular change can be defined as biological changes, seen over a period of time, brought about by environmental changes (Moore & Ross, 2013). Such changes can include differences in health, nutrition, diet, exercise, medical care and climate (Trotter & Gleser, 1951; Tanner, 1989; Meadows & Jantz, 1995; Meadows Jantz & Jantz, 1999; Bogin & Rios, 2003; Stulp & Barrett, 2014). Dissimilarities in environments will have varying effects on populations producing specific differences (Smith, 2007; Cardoso, 2009, Baines et al., 2011), and as such methods derived from

one population are not applicable to other populations (Trotter & Gleser, 1958; Cardoso et al., 2016).

In the case of sub-adults, stature estimation research is obstructed by the lack of large, modern skeletal collections with documented biological information (Telkkä et al., 1962; Sundick, 1977; Stewart, 1979; Kondo et al., 2000; Lewis & Rutty, 2003; İşcan & Steyn, 2013). This has led many to study the skeletal remains of sub-adults from X-rays collected during longitudinal growth studies conducted in the early twentieth century (Feldesman, 1992; Telkkä et al., 1962; Ruff, 2007; Smith, 2007). Unfortunately, many of these studies do not take into consideration the distortion or magnification errors introduced by X-rays (Palkama et al., 1962; Telkkä et al., 1962; Virtama et al., 1962; Smith, 2007) which makes the applicability of these studies suspect.

To overcome the limitations described above, the current study used Magnetic Resonance Imaging (MRI) scans to assess stature estimation in Black South African adult females and sub-adults.

2.5. MAGNETIC RESONANCE IMAGING

MRI is a safe imaging technique that does not involve ionizing radiation (McRobbie et al., 2007). It is frequently used to produce an external and internal image of the human body and was specifically selected for use in this study as it provided the opportunity to study the skeletal remains of living individuals without exposing participants to harmful radiation. Additionally, skeletal data collected from MRI scans is considered accurate and comparable to that collected from CT scanograms and dry bones (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). Measuring skeletal elements from MRI scans also affords the rare opportunity to regress skeletal

measurements against known living stature and allows for the invaluable opportunity to derive more accurate and precise stature estimation equations (Wilson et al., 2010).

Measuring skeletal elements from living participants reduces the unwanted effects of secular trends which is often a problem associated with skeletal collections that have been compiled over a number of decades. It also provides an opportunity for one individual to collect measurements consistently and as such circumvents inter-observer errors, which is major source of error in skeletal research (Ousley, 1995; Krishan et al., 2012c). Measuring stature in living participants in the mornings can also help reduce the effects of the diurnal variation of stature. This decrease in stature is brought about by the compression of cartilage found between joints, especially that of the intervertebral discs (Kobayashi & Togo, 1993; Sjøvold, 2000; Siklar et al., 2005; Krishan & Vij, 2007) and usually varies between 1 – 2 cm, but can be as much as 10 cm (Sjøvold, 2000).

This literature review has provided a succinct synopsis of the current literature available on stature estimation for adult and sub-adults. It also highlighted the problems associated with research in skeletal collections, which the current study aims to circumvent by studying the skeletal remains of living individuals, using MRI scans. Complications regarding the accuracy of stature estimation methods in Black South African adult females and the lack thereof in Black South African sub-adults were also highlighted.

CHAPTER THREE

The Accuracy and Repeatability of Skeletal Measurements Collected from Magnetic Resonance Imaging Scans

3.1. INTRODUCTION

Research on skeletal remains is often hampered by the lack of large, contemporary skeletal collections with known demographic information (İşcan, 2005). This is especially true for research related to sub-adults (Telkkä et al., 1962; Lewis & Rutty, 2003; İşcan & Steyn, 2013), and available adult skeletal collections are often subject to multiple sources of bias (Komar & Grivas, 2008). Additionally, it is unclear how well skeletal collections represent the population from which they have been derived (Komar & Grivas, 2008), with many collections having an over representation of males, Black individuals, the elderly and individuals from lower socio-economic standings (Hunt & Albanese, 2005; L'Abbé et al., 2005; Komar & Grivas, 2008; Dayal et al., 2009). Other pitfalls include estimated demographic parameters, incorrect recordings and missing data (Lundy, 1983; Bidmos, 2005; Hunt & Albanese, 2005; L'Abbé et al., 2005; Komar & Grivas, 2008; Dayal et al., 2009). For these reasons, the applicability of data derived from skeletal collections, which usually represents earlier generations, indicates that this data produces lower accuracies when applied to modern populations, an effect that has been attributed to secular change (Meadows & Jantz, 1995; Ousley & Jantz, 1997; Meadows Jantz & Jantz, 1999; Dirkmaat et al., 2008; Komar & Grivas, 2008).

To overcome these limitations anthropologists have collaborated with radiologists to study the skeletal remains of living populations (İşcan, 2005). This has led to a plethora of publications on the estimation of age-at-death, sex and stature, utilising various imaging modalities, including X-ray (Telkkä et al., 1962; Petrovečki et al., 2007), Computed Tomography (CT) (Ramsthaler et al., 2010; Hishmat et al., 2015), Lodox-statscan (Stull et al., 2013) and Magnetic Resonance Imaging (MRI) (Bidmos & Manger, 2012; Ekizoglu et al., 2015; Guo et al., 2015). As these imaging modalities are becoming increasingly popular, questions regarding the accuracy and

reliability of the osteometric measurements collected from radiographs have increased. As these imaging modalities are becoming increasingly popular, the accuracy and reliability of the osteometric measurements collected from radiographs have been questioned.

Images generated from X-ray are often distorted (Maresh, 1955; Telkkä et al., 1962) and adjustments are required to compensate for the geometric magnification (Feldesman, 1992; Schroeder et al., 1997). In addition, X-ray exposes participants to harmful ionizing radiation which does not ethically justify the use thereof when dealing with healthy, living participants (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012).

A study by Robinson et al. (2008) and Stull et al. (2014b) assessed the accuracy and reliability of osteometric measurements collected from CT scans. Both studies compared osteometric measurements collected from CT scans to the dry bone measurements. Differences between CT and dry bone measurements were noted and ascribed to the difficulties related to the identification of osteometric landmarks commonly used in traditional measurements (Dedouit et al., 2007). Additionally, CT scans are artefact prone (Barrett & Keat, 2004) and, like X-rays, expose participants to radiation (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). Nevertheless, results indicated that CT scans were a valuable means of collecting osteometric data, as measurements were found to be precise and accurate (Robinson et al., 2008; Stull et al., 2014b).

Lodox-statscan emits very low radiation dosages with the additional advantage of rapidly producing (10-13 seconds) high quality images (Evangelopoulos et al., 2009; Stull et al., 2013). Stull et al. (2013) assessed the distortion caused by Lodox-statscan by comparing osteological measurements collected from dry bone scans to physical measurements of the corresponding dry

bones and showed distortion in the slot axis (x-axis) with negligible distortion in the scan axis (y-axis) (Stull et al., 2013).

MRI is not commonly used to assess the skeletal system due the lack of a bone MR signal; however, bones can still accurately be examined based on the MR signal produced by the surrounding tissue (Rathnayaka et al., 2012). It produces images of high quality with good tissue contrast, and due to the lack of ionizing radiation it is a safe alternative to study the musculoskeletal system in the living (Leitzes et al., 2005; Rathnayaka et al., 2012). A few studies have assessed the accuracy of MRI skeletal measurements in various ways. This includes comparing measurements collected from MRI scans of dry bone to measurements collected directly from the dry bones (Leitzes et al., 2005). Another study compared 3D generated bone MRI models of sheep and human cadaveric limbs to defleshed bone models generated with scanners (Rathnayaka et al., 2012). One study compared the MRI measurements collected from a fleshed cadaver lower limb to measurements collected from the defleshed bones (Doyle & Winsor, 2011). Unfortunately the sample included only one cadaver limb and included non-traditional osteological measurements (Doyle & Winsor, 2011).

Assessing the accuracy and reliability of MRI skeletal measurements collected from fleshed specimens compared to dry bone measurements are vital as current forensic anthropological standards have been generated from dry bones (Brough et al., 2013). Therefore, the aim of this study was to assess the accuracy and repeatability of skeletal measurements collected from MRI scans.

3.2. METHODS AND MATERIALS

3.2.1. Specimens

Partly fleshed left and right lower limbs from ten cadavers, housed in the School of Anatomical Sciences, University of the Witwatersrand, were used in the current study. To further increase the sample size an additional 16 previously dissected cadaver lower limbs were also included. The sample presented Black and White South African males (n = 9) and females (n = 11), aged between 48 and 91 years. Any limbs with signs of pathology, bone damage or surgical implants/intervention were excluded from the study. Ethical clearance for research on cadaveric remains housed in the School of Anatomical Sciences has previously been obtained from the Human Research Ethics Committee – Medical, University of the Witwatersrand (Clearance certificated number: W-CJ-140604-1). Specimens that presented with dried soft tissue were soaked in a formalin bath for one to four days and allowed to air dry. All specimens were sealed in labelled plastic bags and transported to the Department of Radiology, Wits-Donald Gordon Medical Centre, Johannesburg, South Africa for scanning.

3.2.2. Methods

3.2.2.1. Magnetic resonance imaging (MRI)

MRI scanograms were taken using a 1.5 T Phillips Entera MR scanner, Release 3.2 level 2 2011-01-11 CE 0344 with software version 12.1. The limbs were placed in the anatomical position with the aid of blocks. A T1-weighted survey scan was completed, TR between 3000-4000, using 130 mm slice thickness in a sagittal sequence. This was followed by a T2-weighted MOBI track scan from the head of the femur to the foot using three stations and a 3mm coronal

sequence. The three MOBI track stations were fused on a working station and the images were saved as DICOM (Digital Imaging and Communication in Medicine) files.

3.2.2.2. Bone maceration and degreasing

Following the MRI scans all the joints of the limbs, except those of the foot, were dis-articulated and the associated soft tissue was manually removed. Standard anthropometric measurements, as listed below, were collected from the wet bones using a sliding caliper and an osteometric board. Thereafter the bones were macerated and degreased following the standard operating procedure of the School of Anatomical Sciences, University of the Witwatersrand. Maceration involved boiling the bones in water with an added detergent for three consecutive days. Any adhering soft tissue was removed with scalpels and/or scourer pads. Finally the bones were degreased in a trichloroethylene pot (defatting plant) for 2-3 days at 82°C and allowed to dry for up to five days. Once dry the bones were bleached with hydrogen peroxide and ammonia for a week and left to dry for between five to ten days after which anthropometric measurements were collected.

3.2.2.3. Anthropometric measurements

Standard anthropometric measurements were collected according to descriptions by Moore-Jansen et al. (1994), Raxter et al. (2006), Bidmos & Manger (2012) and Brough et al. (2013). The image processing software OsiriX was used to manage, manipulate and measure the MRI scans (Rosset et al., 2004).

The following measurements were collected:

1. Maximum length of the femur: the maximum distance between the head of the femur and the medial condyle (Figure 3.1).
2. Physiological (bicondylar) length of the femur: the distance between the femoral head and a plane drawn along the inferior aspects of the femoral condyles (Figure 3.1).
3. Transverse mid-shaft femur diameter: the medio-lateral diameter of the femur taken perpendicular to the long axis of the bone, at the mid-shaft (Figure 3.2).
4. Maximum femoral head diameter: maximum distance between the articular surfaces of the head of the femur (Figure 3.2).
5. Epicondylar breadth of the femur: the breadth of the femur measured between the most protruding aspects of the epicondyles (Figure 3.3). Due to adhering ligaments and other soft tissue associated with the epicondyles of the femur, this measurement was not collected on the wet specimens.
6. Length of the tibia (condylomalleolar length): the distance between the superior margin of the lateral condyle and the most inferior aspect of the medial malleolus (Figure 3.4).
7. Maximum proximal epiphyseal breadth of the tibia: the breadth measured between the most protruding aspects of the medial and lateral tibial condyles (Figure 3.3). Similar to the epicondylar breadth of the femur this measurement was not collected on the wet specimens due to adhering tissue.
8. Ankle (talo-calcaneal) height: the articulated height of the talus and calcaneus was measured from the most superior aspect of the talus to the most inferior aspect of the calcaneal tuberosity. Two measurements were collected from the MRI scans to assess the accuracy thereof. The first was a lateral ankle height measurement taken in accordance

with descriptions by Dayal et al. (2008) in a sagittal view (Figure 3.5A). The second measurement was a posterior ankle measurements collected following stipulations by Bidmos & Manger (2012) in a coronal view (Figure 3.5B).

To ensure that maximum measurements were collected from the MRI scans, sequential slices portraying the most proximal and distal as well as the most medial and lateral osteometric landmarks were merged prior to data collection (Brough et al., 2013).

3.2.3. Data analysis

A Lin's (1989) concordance correlation coefficient of reproducibility was used to assess intra- and inter-observer error. Ten randomly selected lower limbs were measured and re-measured by the initial observer as well as an independent observer, during each of the assessment phases.

All statistical analyses were conducted in SPSS, version 22. Descriptive statistics including the mean and standard deviations were calculated for all variables. As dry bones are most commonly used for osteological research and assessed during forensic anthropological cases, it was used as the "gold standard" for comparisons as suggested by Leitzes et al. (2005), Robinson et al. (2008) and Brough et al. (2013). A paired sample t-test was used to compare all dry bone measurements to that collected from the MRI scans and wet bones. This was done to establish if any differences existed between the measurements. In cases where no significant differences were observed, the agreement of the measurements was assessed using Bland-Altman plots. These plots visually illustrate the agreement between methods (Bland & Altman, 1986; Myles & Cui, 2007). One sample t-tests were performed to investigate the presence of any systemic differences (fixed

bias) between the measurements while proportional bias was assessed using linear regression analyses.

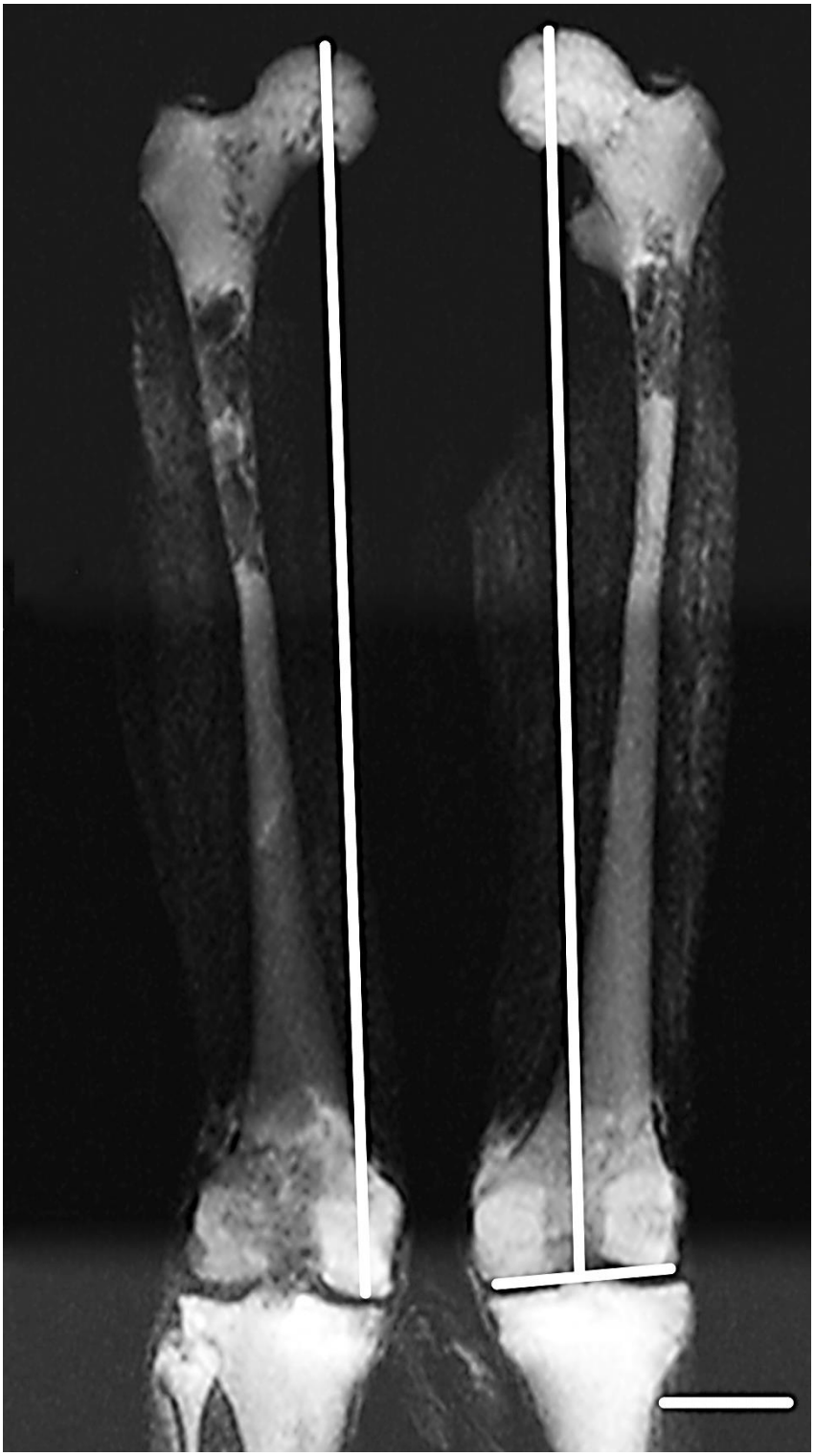


Figure 3.1. A coronal MRI scanogram illustrating the maximum (left) and physiological (right) length of the femur; scale = 5 cm.



Figure 3.2. Image illustrating the maximum diameter of the head (left) as well as the transverse mid-shaft diameter of the femur (right); scale = 5 cm. (Please note that the coronal scanogram in this image is not situated through the maximum transverse diameter of the femur mid-shaft.)

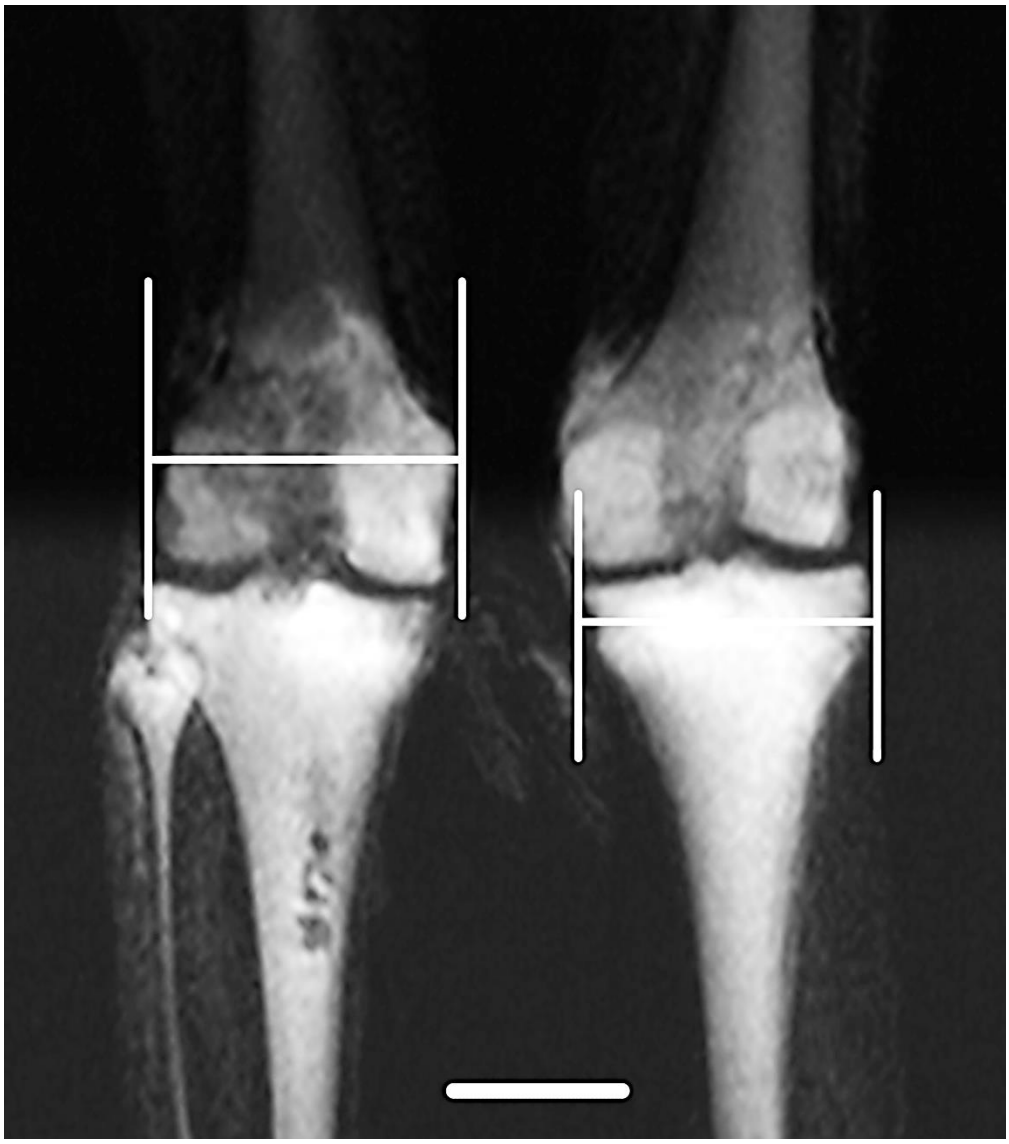


Figure 3.3. Image illustrating the epicondylar breadth of the femur (left) along with the maximum proximal epiphyseal breadth of the tibia (right); scale = 5 cm.

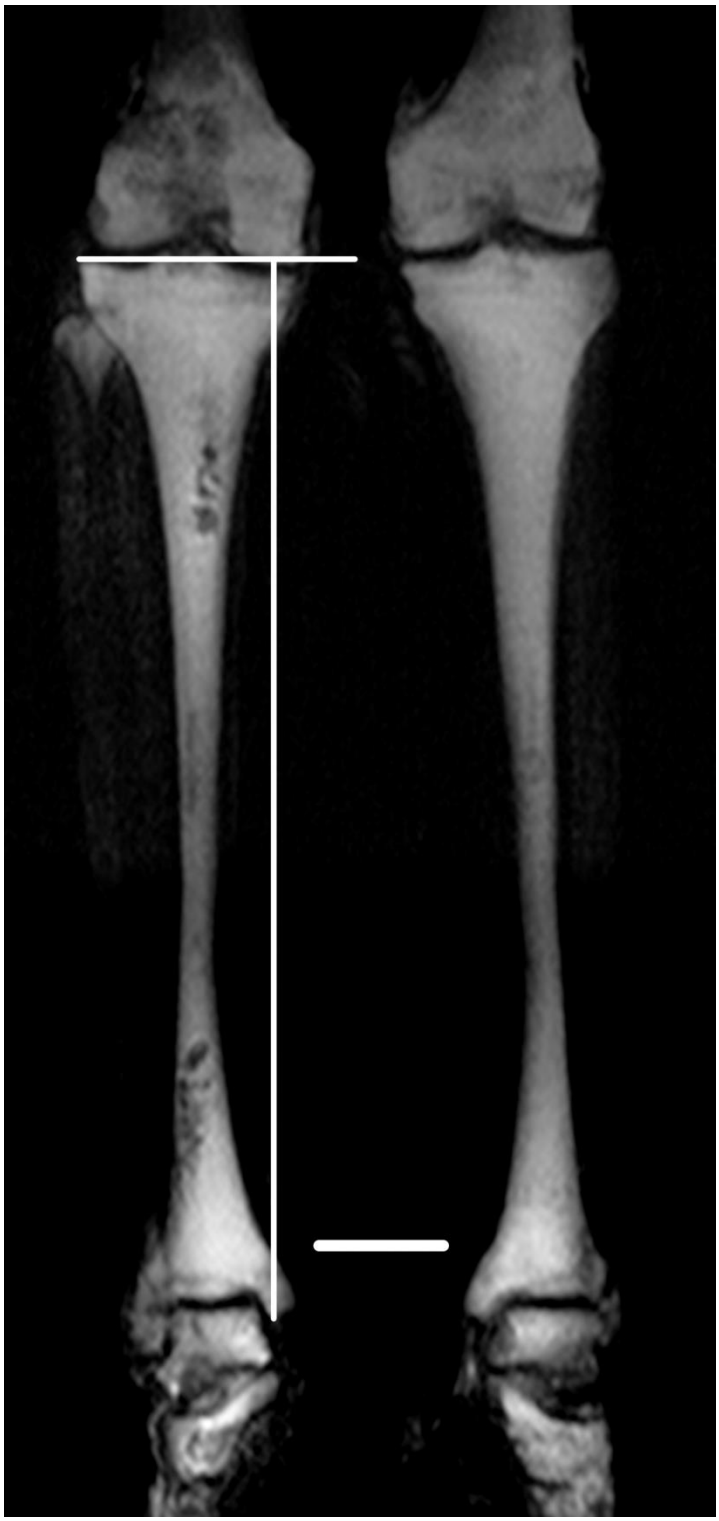


Figure 3.4. Image illustrating the condylomalleolar length of the tibia; scale = 5 cm.

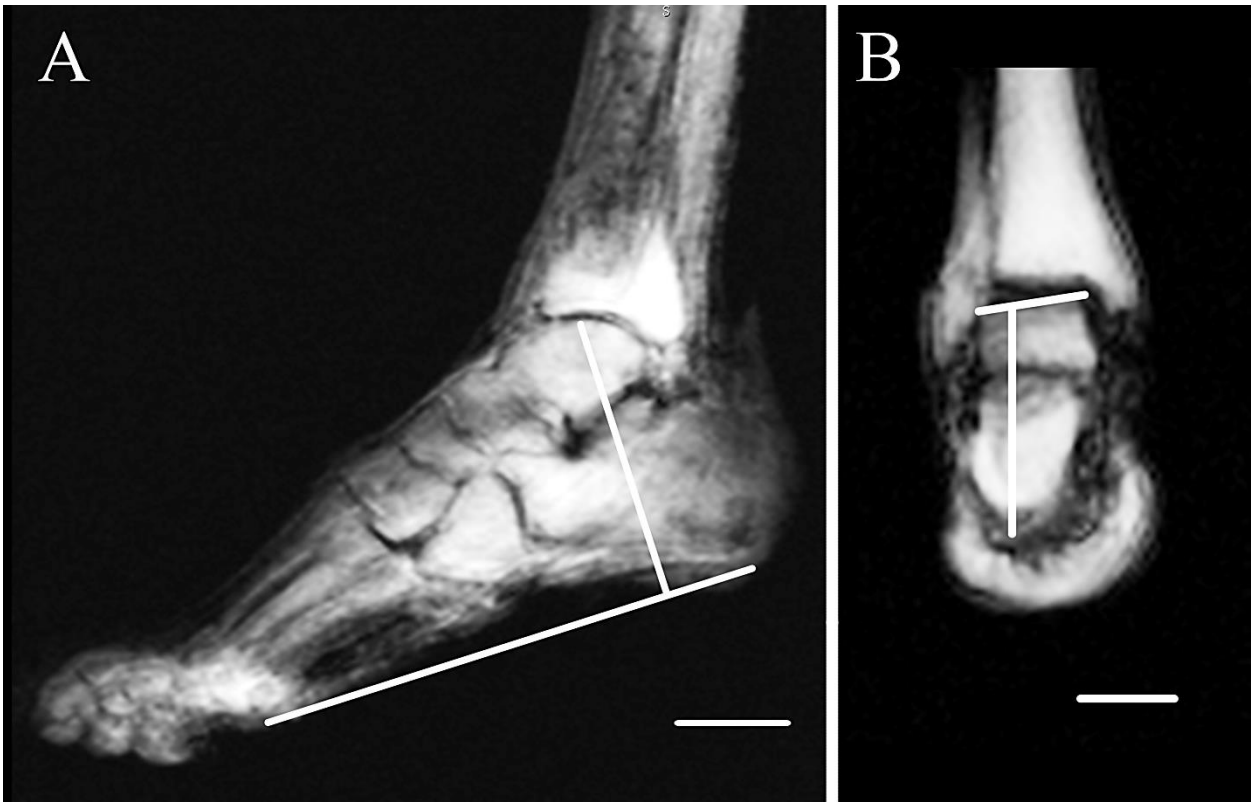


Figure 3.5. MRI scanogram illustrating the A) lateral ankle height measurements collected in a sagittal view according to descriptions by Dayal et al. (2008) and B) the posterior measurements collected in a coronal view following stipulations by Bidmos & Manger (2012); scale = 3 cm.

3.3. RESULTS

3.3.1. Repeatability

The results of the Lin's concordance correlation coefficient of reproducibility for both the intra- and inter-observer repeatability are summarised in Table 3.1. All wet and dry bone measurements had Pc-values greater than 0.90, indicating very high intra- and inter-observer repeatability (Lin, 1989). Similarly all MRI measurements had Pc-values greater than 0.90 except for the intra- and inter-observer repeatability of the posterior ankle height measurements (Pc = 0.85 and Pc = 0.82, respectively). The inter-observer repeatability of the epiphyseal breadth of the proximal tibia also had a Pc-value just below 0.90 (Pc = 0.87).

3.3.2. Descriptive statistics

The means and standard deviations for all dry, wet and MRI bone measurements are summarised in Table 3.2. The epicondylar breadth of the femur and the proximal epiphyseal breadth of the tibia were not collected from wet bones due to adhering soft tissue, which could not be manually removed.

The mean osteological measurements collected from dry, wet and MRI bones were very similar, however, all wet bone measurements were slightly larger than the MRI bone measurements, followed by the dry bone measurements. The difference in sample size for measurements using MRI scans is due to the exclusion of a number of poor quality MRI scans and other technical errors.

3.3.3. Dry vs. wet bone measurements

The mean difference between dry bone and wet bone measurements, along with the results of the paired t-tests are reported in Table 3.3. All wet bone dimensions were overestimated compared to dry bone measurements, with overestimations ranging between 0.1 cm and 1.5 cm.

Results from the paired t-test indicated statistically significant differences between dry bone measurements and that collected from wet bones and as such the wet bone measurements are not similar to that collected from dry bones.

3.3.4. Dry vs. MRI bone measurements

The mean difference between dry bone and MRI measurements, along with the results of the paired and one sample t-tests are summarised in Table 3.3. All MRI and dry bone measurements were very similar, with no difference found between the average femur midshaft and head diameter measurements. The MRI tibial length and lateral ankle height measurements were slightly overestimated compared to dry bone measurements, with overestimations ranging between 0.04 cm and 0.11 cm. All other MRI measurements were marginally underestimated compared to dry bone measurements, with underestimations ranging between 0.03 cm and 0.23 cm.

The mean difference between dry bone and MRI measurements showed no statistically significant differences ($p>0.05$), except for the epicondylar breadth of the femur. Likewise, one sample t-tests showed that the mean difference of measurements between dry bones and MRI scans were not significantly different from 0; indicating that these measurements are the same and showed no fixed bias.

To further assess the agreement between the measurements, Bland-Altman plots were generated (Figures 3.6-3.13). The differences between the measurements were randomly scattered around zero. Moreover, the majority of the scatter, representing the difference between the dry bone and MRI measurements, fell within the upper and lower levels of agreement.

Regression analyses (Table 3.3) confirmed no proportional bias between dry bone and MRI measurements ($p>0.05$), except for the ankle height measurements. This bias is, however, likely due to the small sample size available for analysis.

3.3.5. MRI ankle height measurements

The ankle height measured from the posterior view (coronal sequence) from MRI scans was smaller than the ankle height measurements collected from the lateral view (sagittal sequence). These differences were statistically significant ($p = 0.002$). The mean difference between the dry bone ankle height measurement and the MRI measurements were -0.11 cm for the lateral and 0.23 cm for the posterior measurements, with the posterior measurement underestimating and the lateral measurement overestimating the dry bone ankle height measurement. The differences between the dry bone and, lateral and posterior MRI ankle height measurements, as illustrated in Table 3.3 were, however, not statically significant ($p = 0.267$ and $p = 0.062$, respectively).

Table 3.1. Lin's concordance correlation coefficients of reproducibility (Pc-values) for intra- and inter-observer repeatability.

	Intra-observer			Inter-observer		
Variables	**MRI**	**Wet**	**Dry**	**MRI**	**Wet**	**Dry**
Maximum femur length	0.99	0.99	0.99	0.99	0.99	0.99
Physiological femur length	0.99	0.99	0.99	0.99	0.99	0.99
Femur midshaft diameter	0.98	0.98	0.99	0.94	0.99	0.99
Femoral head diameter	0.95	0.99	0.96	0.90	0.98	0.91
Femur epicondylar breadth	0.95	-	0.96	0.92	-	0.96
Tibial length	0.99	0.99	0.99	0.99	0.98	0.99
Epiphyseal breadth of tibia	0.96	-	0.96	0.87	-	0.96
Ankle height (lateral)	0.96	0.91	0.95	0.92	0.97	0.96
Ankle height (posterior)[a]	0.85	-	-	0.82	-	-

[a]*The posterior ankle height as described by Bidmos & Manger (2012) was only measured from MRI scans so as to compare the accuracy thereof with the standard anthropometric measurement (lateral ankle height)*

Table 3.2. Descriptive statistics, including the mean and standard deviation (SD) of the MRI, wet bone and dry bone measurements.

	Dry bone		Wet bone		MRI scans	
Variables (cm)	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**n**	**Mean ± SD**
Maximum femur length	36	46.5 ± 2.7	36	46.9 ± 2.7	32	46.7 ± 2.7
Physiological femur length	36	46.2 ± 2.7	36	46.5 ± 2.7	29	46.4 ± 2.7
Femur midshaft diameter	36	2.7 ± 0.2	36	2.8 ± 0.2	23	2.7 ± 0.2
Femoral head diameter	36	4.6 ± 0.4	36	4.8 ± 0.4	31	4.7 ± 0.4
Femur epicondylar breadth	36	8.2 ± 0.5	-	-	35	8.0 ± 0.5
Tibial length	36	38.1 ± 2.3	36	38.7 ± 2.3	34	38.3 ± 2.3
Epiphyseal breadth of tibia	36	7.5 ± 0.5	-	-	36	7.4 ± 0.5
Ankle height (lateral)	35	7.0 ± 0.4	35	8.5 ± 0.5	16	7.1 ± 0.6
Ankle height (posterior)	-	-	-	-	30	6.7 ± 0.6

Table 3.3. Summary of the mean difference between dry bone measurements and that collected from wet bones and MRI scans, along with the results for the t-tests (paired and one sample) and regression analyses (p-value).

	Dry vs. Wet		Dry vs. MRI			
Variable	**Mean difference**	**Paired t-test**	**Mean difference**	**Paired t-test**	**One sample t-test**	**Regression**
Maximum femur length	-0.36	0.021	0.04	0.263	0.263	0.829
Physiological femur length	-0.30	0.032	0.03	0.830	0.830	0.456
Femur midshaft diameter	-0.09	0.001	-0.00	0.984	0.984	0.845
Femoral head diameter	-0.22	0.0001	0.00	0.973	0.973	0.111
Femur epicondylar breadth	-	-	0.14	0.002	-	-
Tibial length	-0.59	0.0001	-0.04	0.273	0.273	0.850
Epiphyseal breadth of tibia	-	-	0.06	0.164	0.164	0.652
Ankle height (lateral)	-1.50	0.0001	-0.11	0.267	0.267	0.008
Ankle height (posterior)	-	-	0.23	0.062	0.062	0.037

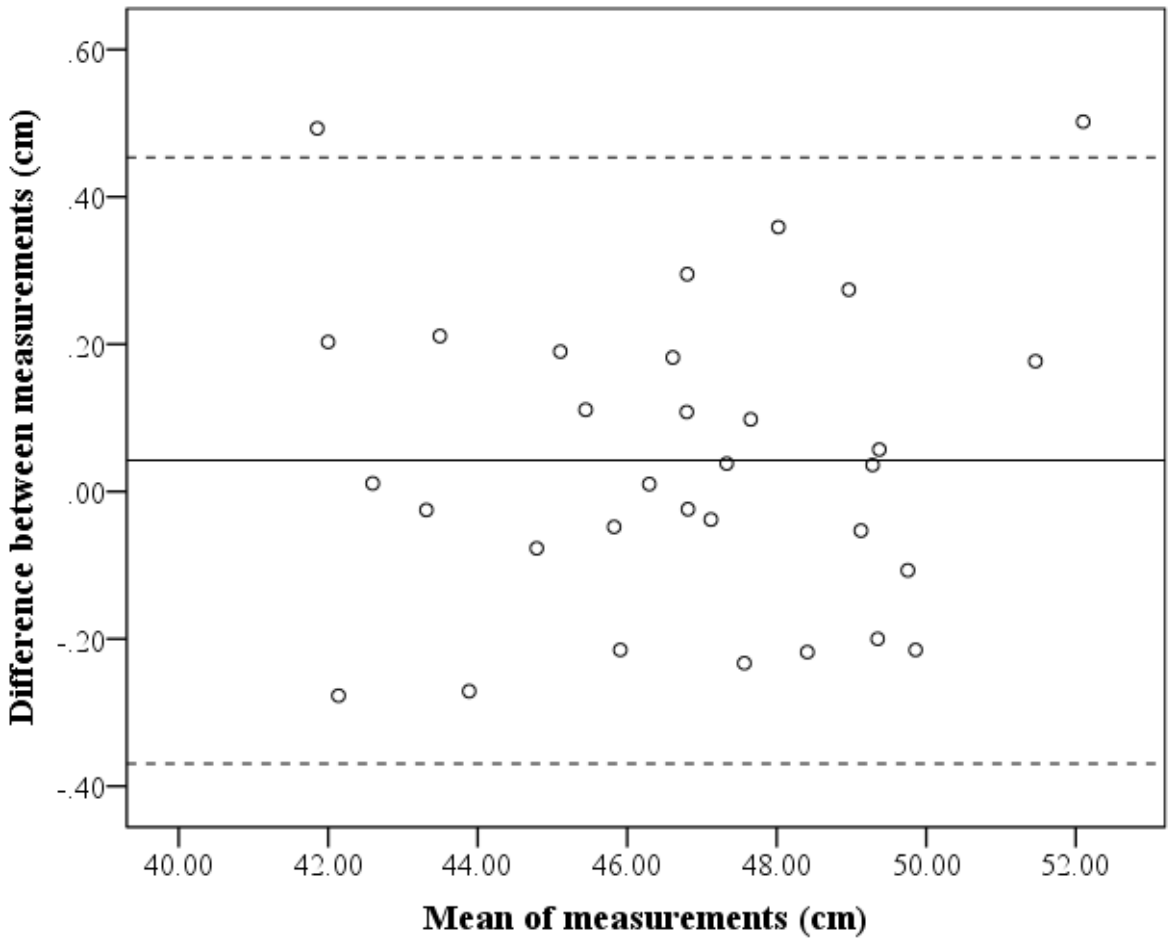


Figure 3.6. Bland-Altman plot illustrating the agreement of the maximum length of the femur measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

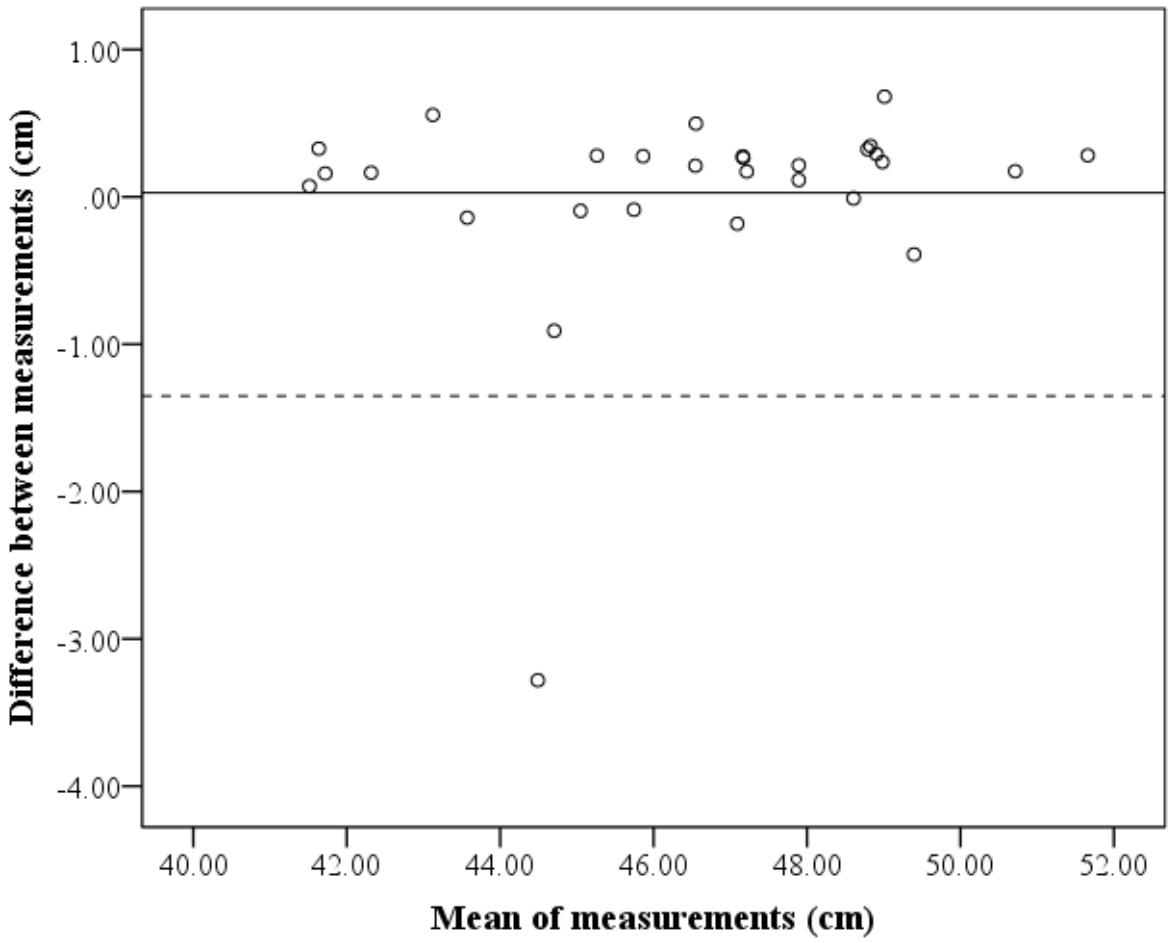


Figure 3.7. Bland-Altman plot illustrating the agreement of the physiological length of the femur measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

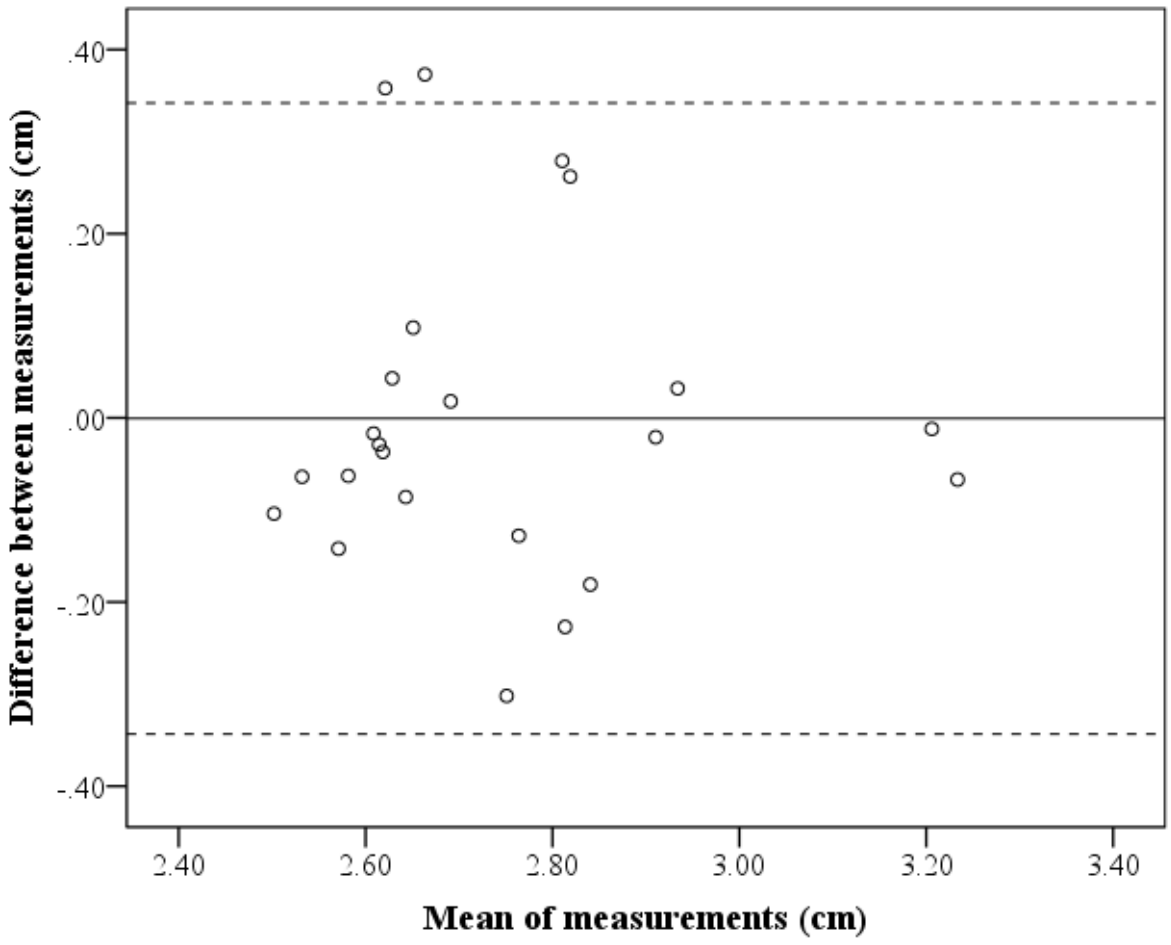


Figure 3.8. Bland-Altman plot illustrating the agreement of the transverse midshaft femur diameter measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

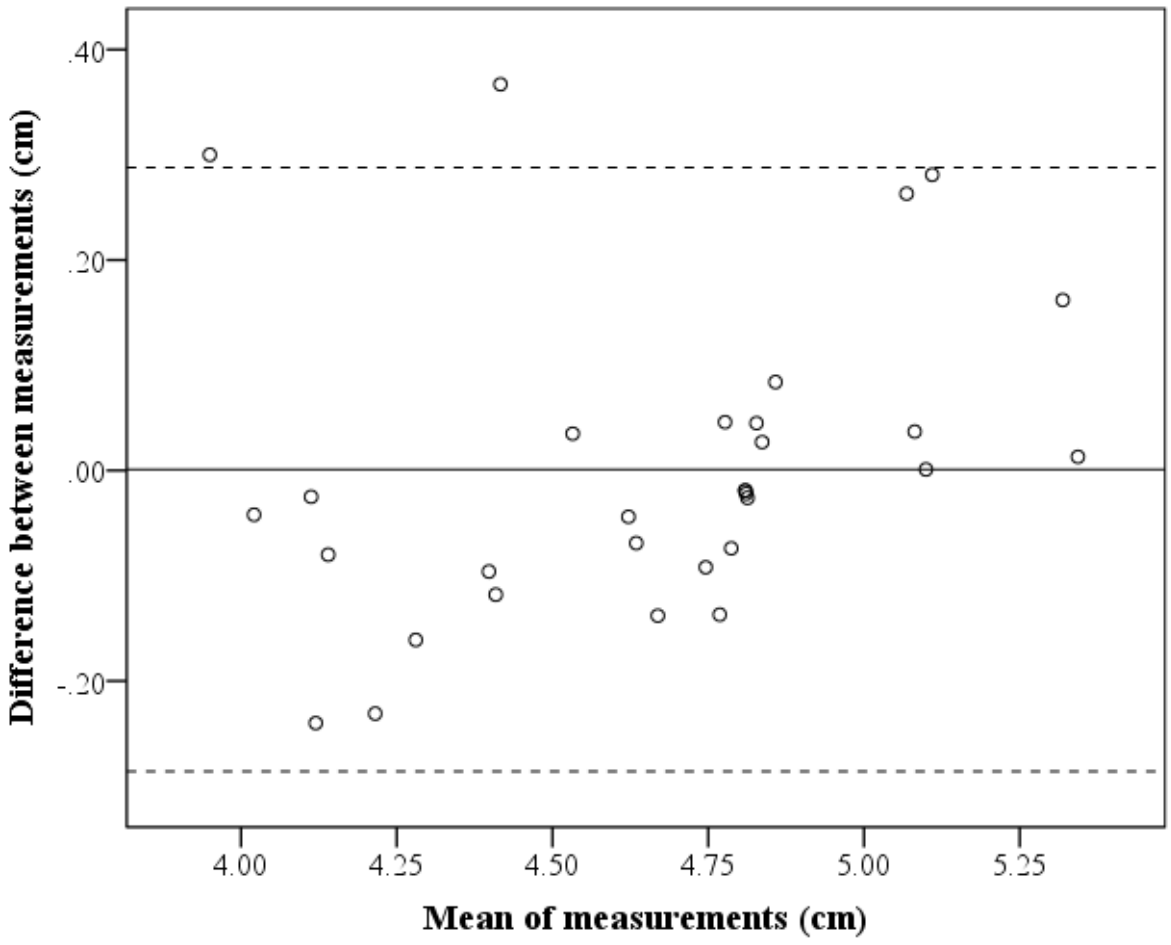


Figure 3.9. Bland-Altman plot illustrating the agreement of the maximum femoral head diameter measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

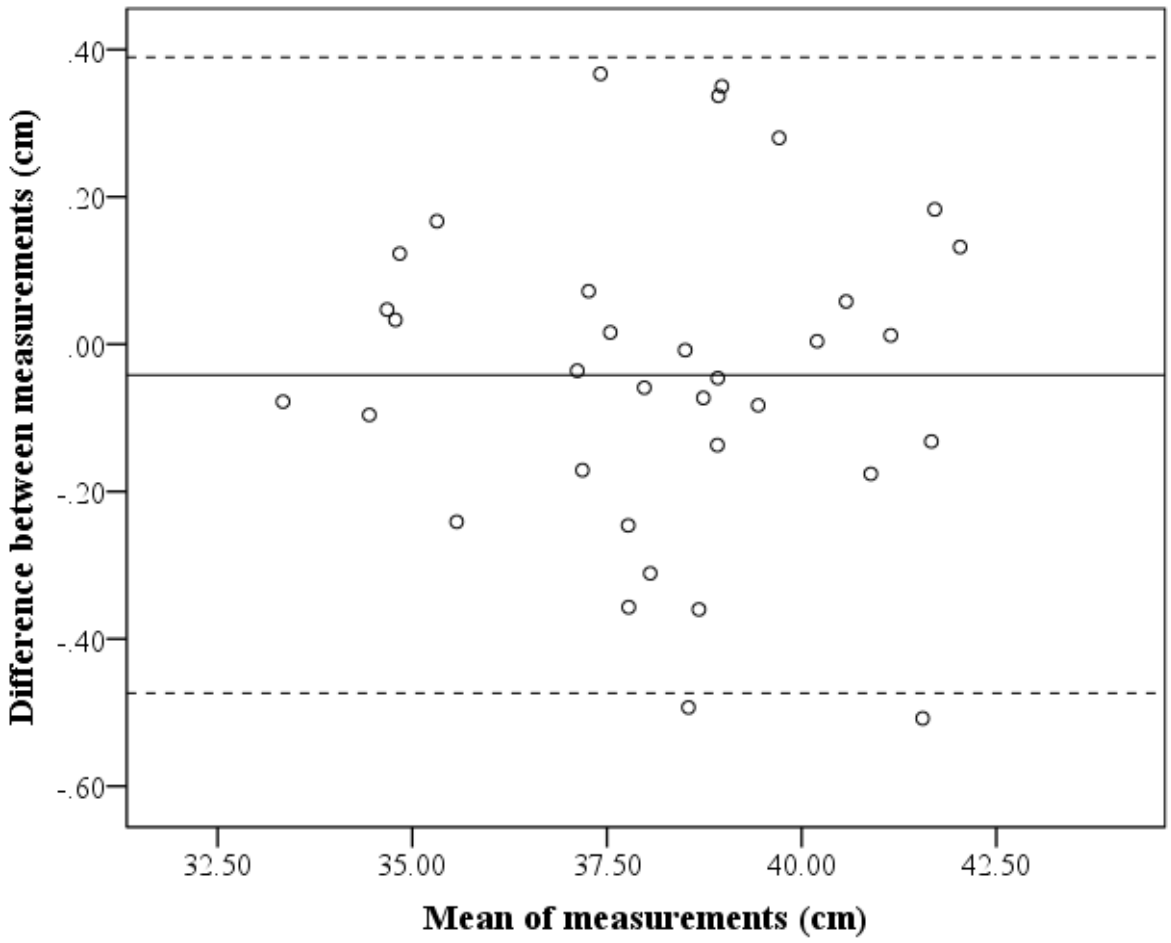


Figure 3.10. Bland-Altman plot illustrating the agreement of the length of the tibia (condylomalleolar length) measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

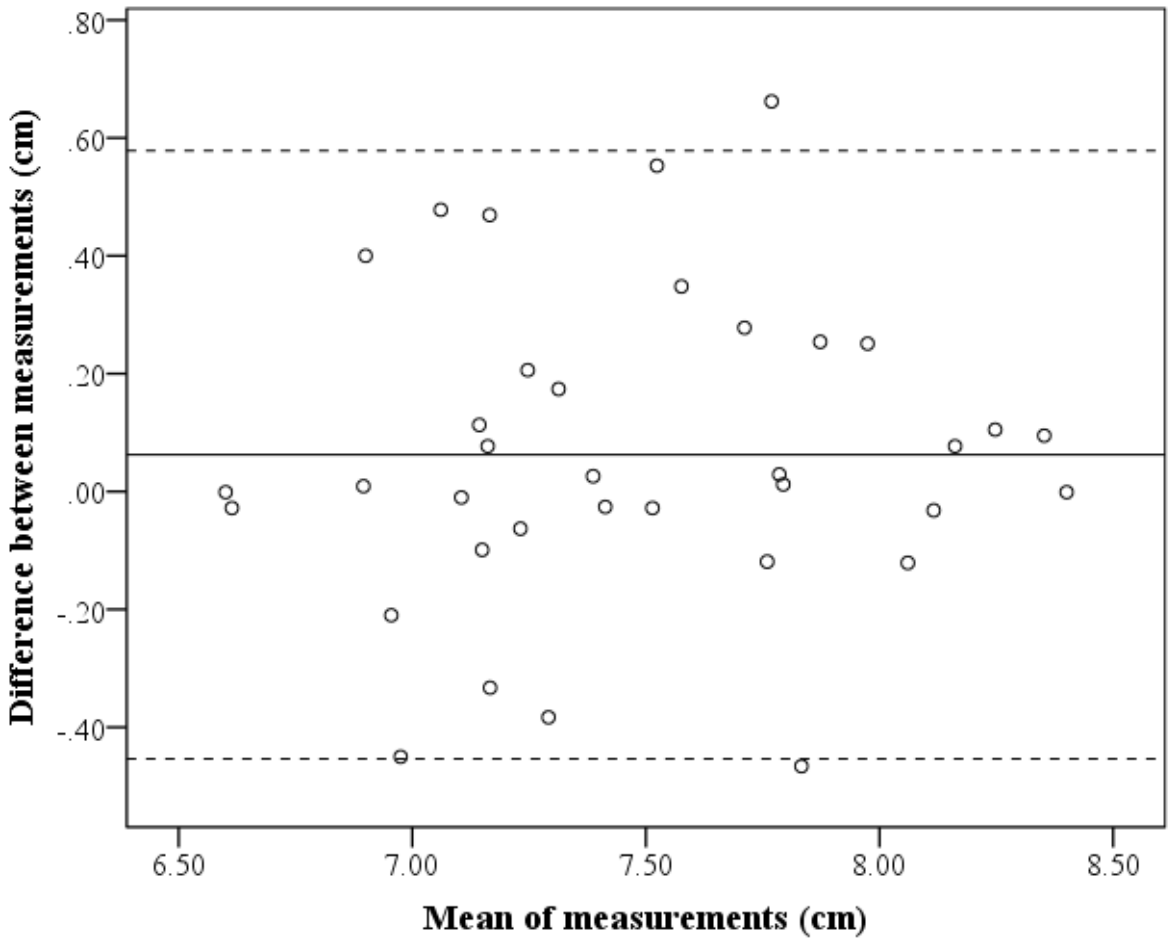


Figure 3.11. Bland-Altman plot illustrating the agreement of the maximum proximal epiphyseal breadth of the tibia measurements collected from dry bones and MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

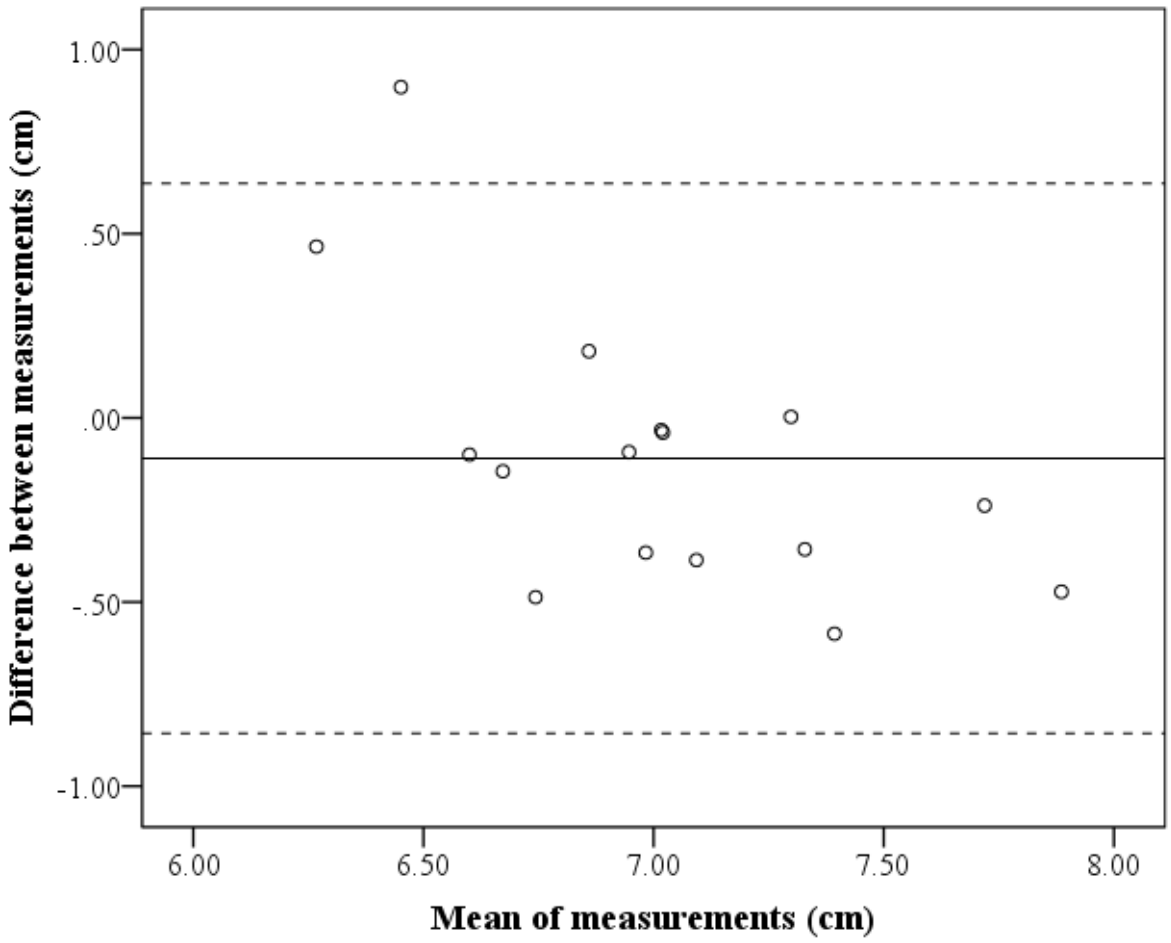


Figure 3.12. Bland-Altman plot illustrating the agreement of the ankle height measurements collected from dry bones and on lateral MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

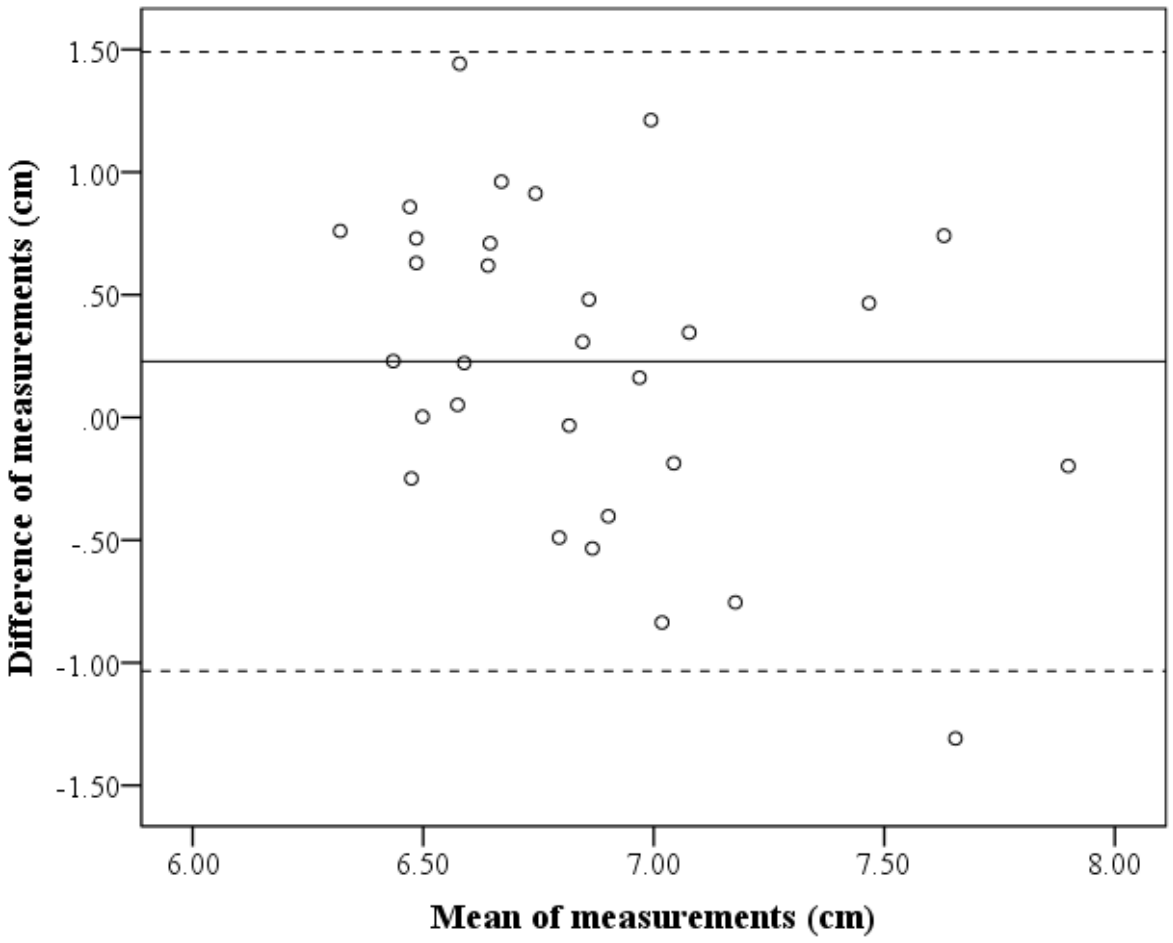


Figure 3.13. Bland-Altman plot illustrating the agreement of the ankle height measurements collected from dry bones and on posterior MRI scanogram along with the limits of agreement (95% confidence intervals; dashed line).

3.4. DISCUSSION

The lack of skeletal collections along with the bias associated with numerous current collections has resulted in an increase in research utilising various imaging techniques. These imaging modalities might bridge the problems related to skeletal collections, but brings about questions regarding the accuracy of the skeletal measurements collected from such images (Cardoso et al., 2016). Therefore the aim of the current study was to assess whether bone measurements collected from MRI scans are repeatable and secondly to evaluate if the skeletal measurements collected from MRI scans are comparable to those collected from dry bones.

3.4.1. Repeatability

Based on the large Pc-values all measurements were deemed highly repeatable. These high repeatability values re-affirm clear measurement definitions and ease of data collection, whether directly from bone or from the MRI scanograms. Likewise, high repeatabilities for osteological data collected from MRI were also reported by Leitzes et al. (2005), Doyle & Winsor (2011) and Bidmos & Manger (2012).

Lower repeatability values were, however, noted for the epiphyseal breadth of the tibia and posterior ankle height measurements, collected from MRI scans. The lower inter-observer repeatability value noted for the epiphyseal breadth of the tibia could be associated with the observer's experience or the lack of sufficient surrounding soft tissue. Bones do not generate a MR signal and as such the quality of bone MRI scans rely on the MR signal produced by the soft tissue surrounding the bone (Rathnayaka et al., 2012). Unfortunately the current study had to include a number of previously dissected cadaver limbs, often devoid of sufficient surrounding soft tissue, to increase the sample size. This lack of soft tissue might have compromised the quality of the

MRI scan along with the geometric outline of the bone to be measured and as such caused a decrease in the measurement repeatability.

The reduced repeatability observed for the posterior ankle height measurements collected from MRI scans are ascribed to the difficulties related to identifying the osteological landmarks required to accurately measure the ankle height. This is because the posterior ankle height measurement was obtained from a coronal MRI sequence which does not permit a complete view of the articulated talo-calcaneal complex (Bidmos & Manger, 2012).

3.4.2. MRI ankle height measurements

The precision of the ankle height measurement described by Bidmos & Manger (2012) was questioned by Ruff et al. (2012) and an assessment of the accuracy was warranted. The mean posterior ankle height measurement (6.7 ± 0.6 cm) collected from the MRI scans, according to the definition by Bidmos & Manger (2012) was smaller than the ankle height measurement collected from dry bones (7.0 ± 0.4 cm). As suspected by Ruff et al. (2012) the posterior ankle height measurement underestimated the true value of the articulated talo-calcaneal height collected from dry bones (0.23 cm), however, these differences were not statistically significant ($p = 0.062$). Additionally, the magnitude of the underestimation (0.23 cm) was substantially smaller than that proposed by Ruff et al. (2012) (2 cm). The repeatability of the posterior ankle height measurement did cause some concern, as discussed above, due to the difficulties associated with the identification of the osteological landmarks. It was therefore proposed that a lateral ankle height measurement be taken from sagittal MRI scans. This measurement is better aligned with the articulated height of the talus and calcaneus collected from dry bones, as defined by Dayal et al. (2008). Hence to accurately measure the ankle height the plane of support was identified on the

MRI scans and the height measured from the most superior aspect of the trochlea perpendicular to this plane. In doing so the calcaneus is kept in the correct inclination angle, regardless of the position of the feet in relation to the body, which was a concern raised by Bidmos & Manger (2012).

The lateral MRI ankle height measurement (7.1 ± 0.6 cm) overestimated the dry bone measurement by 0.11 cm. This measurement did not differ significantly from the dry bone measurement ($p = 0.267$) but did differ from the posterior ankle height measurement ($p = 0.002$). The small overestimation observed in the lateral ankle height measurement is ascribed to the presence of cartilage found between the talus and calcaneus in the living, as is seen in Figure 3.5A. A much larger difference was found between the lateral dry bone ankle height measurement and the wet bone measurement (-1.5 cm). This large difference was due to the presence of cartilage on both the superior and inferior surfaces of the talus and calcaneus along with excessive soft tissue from surrounding ligaments and tendons which could not manually be removed without causing damage to the bones. Apart from better approximating the dry bone ankle height measurement, the lateral MRI ankle measurement also showed an increased repeatability. This is related to the ability to view the entire talo-calcaneal complex and as such allows for the clear identification of all osteological landmarks required to accurately collect this measurement.

3.4.3. MRI accuracy

The osteological measurements collected from dry bone, wet bone and MRI scans were very similar; however, as expected wet bone measurements were significantly larger than that of dry bones. These differences are ascribed to the presence of cartilage and adhering soft tissue which could not be manually removed without damaging the bone. Additionally, differences can

also be related to the shrinkage of bone which has been associated with maceration, degreasing and drying (Ingalls, 1927; Todd & Pyle, 1928; Lievers et al., 2007). The magnitude of this shrinkage is, however, not uniform and is futhermore affected by the amount of compact bone *vs* cancellous bone, presence of soft tissue, drying temperature and time lapse since maceration (Ingalls, 1927; Todd & Pyle, 1928; Lievers et al., 2007).

Differences were also found between dry bone and MRI measurements, however, these differences were not statistically significant, except for the epicondylar breadth of the femur. This difference can be related to the lack of sufficient soft tissue surrounding the distal epiphysis of the femur that is required to produce an MR signal necessary for a clear image (Leitzes et al., 2005). Additionally, poor geometric outlines are often found at the epiphyses of long bones. This is related to the presence of multiple soft tissue types (i.e. muscles, ligaments, cartilage, fat, etc.) attached to bone epiphyses which produce different MR signals. This causes a voxel to carry information related to various tissue types which subsequently leads to the loss of information and ultimately unclear geometric outlines (Rathnayaka et al., 2012).

Distortion errors are often found in the slot axis (x-axis) (Robinson et al., 2008; Stull et al., 2013) of radiographs and are associated with the object-image distance (OID) and source-image distance (Maresh & Deming, 1939; Maresh, 1970; Stull et al., 2013). The mean difference between dry bone and MRI measurements collected in the slot axis, i.e., the femoral head diameter and midshaft diameter of the femur in the current study, showed no difference and serves to supports the accuracy of osteometric measurements collected from MRI scans.

Unfortunately only a few studies are available for direct comparisons and are further reduced by reporting non-standard anthropological measurements. Leitzes et al. (2005) and Doyle & Winsor (2011) compared the femoral length of cadaveric lower limbs collected from MRI scans

and corresponding dry bones and found a mean difference of 2.90 mm and 0.01 cm, respectively. These differences are in good agreement with the differences found in the current study. The errors observed in the current study are also comparable to mean differences reported between dry bone and Lodox-statscan measurements (Stull et al., 2013) as well as dry bone and CT scan measurements (Brough et al., 2013; Hishmat et al., 2015). All mean differences recorded in the current study were less than 2 mm which is within the range of error accepted by forensic anthropologists (Stull et al., 2013, 2014b).

3.5. CONCLUSION

Virtual anthropology has become increasingly popular as it provides a non-invasive means to acquire osteological data without the need for cleaning the remains. It affords one the opportunity to study the skeletal remains of the living without exposing them to harmful radiation, which in turn circumvents problems associated with bias found in skeletal collections (Leitzes et al., 2005; Dedouit et al., 2007; Robinson et al., 2008; Doyle & Winsor, 2011; Bidmos & Manger, 2012; Rathnayaka et al., 2012; Baglivo et al., 2013; Higginbotham-Jones & Ward, 2014). Notwithstanding these advantages, the application of these image modalities in skeletal research has been hampered by questions regarding the accuracy of skeletal measurements collected from such imaging modalities. Therefore the current study aimed to assess the accuracy of lower limb skeletal measurements collected from MRI scans. Results indicated no statistically significant differences between dry bone measurements and that collected form MRI scans. Additionally, the mean differences observed were within the range of error accepted by forensic anthropologist (Stull et al., 2013, 2014b) and as such supports the use of Magnetic Resonance Imaging scans in skeletal research.

To further improve the accuracy and reliability of osteometric data collected from MRI scans, future studies, aimed at the development of protocols for the collection of osteometric from radiological material are encouraged.

CHAPTER FOUR

The Accuracy of the Anatomical Method for Stature Estimation in Black South African Females

4.1. INTRODUCTION

The anatomical method, commonly referred to as Fully's method (Fully, 1956), has repeatedly been described as the most accurate method for stature estimation (Lundy, 1985, 1988; Ousley, 1995; Bidmos, 2005; Maijanen, 2009). This is due to the inclusion of the height measurements of all the bones that contribute directly to stature and as such eliminates the inaccuracies introduced by differing body proportions, which is one of the main sources of error of the mathematical method (Lundy, 1985; Raxter et al., 2006; Maijanen, 2009). The anatomical method is also often used to calculate skeletal height. Subsequently regressions equations are generated for stature estimation from individual bones using the calculated skeletal height, when documented statures are not available (Lundy, 1985, 1988). This approach has been used widely in South Africa as cadaveric heights in skeletal collections are often missing (Bidmos, 2005; Dayal et al., 2008) or inaccurate (Lundy, 1983; Bidmos, 2005).

The accuracy of the anatomical method has previously been tested (Lundy 1988), but results from recent studies have questioned the accuracy of this method as it has been shown that Fully's method continuously underestimates stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Maijanen, 2009; Bidmos & Manger, 2012). It has been suggested that the underestimation is related to the use of the universally applied soft tissue correction factor described by Fully (1956) (King, 2004; Bidmos, 2005; Raxter et al., 2006). King (2004) and Bidmos (2005) proposed that the soft tissue correction factor might be population specific, as the observed inaccuracies were more distinct in Black individuals compared to Whites. Bidmos (2005) furthermore suggested that the soft tissue correction factor might also be sex specific as inaccuracies were more pronounced in Black females compared to Black males. In contrast, Raxter et al. (2006) found that sex and ancestry had no significant effect on the accuracy of stature estimation using the anatomical

method. Age on the other hand was found to be a contributing factor. Raxter et al. (2006) also proposed that the underestimations of the anatomical method could in part be related to the vague definitions used to collect the relevant skeletal measurements, as these measurements were not explicitly defined by Fully (1956). A modified anatomical method, based on regression analysis which incorporated a single soft tissue value as well as the effects of age on stature estimation, was put forward. The authors also provided an equation for stature estimation when age is not available (Raxter et al., 2006).

Using the soft tissue correction factors proposed by Fully (1956) and Raxter et al. (2006), Bidmos & Manger (2012) assessed the accuracy of the anatomical method in living Black South African males. The authors found that both Fully's (1956) and the revised method by Raxter et al. (2006) significantly underestimated stature, on average by 15.8 cm and 14.8 cm respectively, in this population group. A new soft tissue correction factor of 25.9 cm was calculated for the Black South African males; a value that was significantly larger than the previously proposed values of 10.5-11.5 cm by Fully (1956) and 14.6 cm by Raxter et al. (2006). Bidmos & Manger (2012) proposed that these differences were due to the population specificity of the soft tissue correction factor, as earlier suggested by King (2004) and Bidmos (2005). The authors also suggested that the soft tissue correction factor could be sex specific. Therefore the aim of this study was to assess the accuracy of the various soft tissue correction factors of the anatomical method, in a living population of Black South African females and to subsequently calculate a new soft tissue correction factor specific for stature estimation in this population group.

4.2. MATERIALS AND METHODS

4.2.1. Participants

A plethora of stature estimation publications have been generated from skeletal remains housed in various skeletal collections. These collections are often no longer representative of the current living population due to the effect of secular trends (Meadows & Jantz, 1995; Meadows Jantz & Jantz, 1999; Dirkmaat et al., 2008; Wilson et al., 2010). Therefore, ethical clearance was obtained from the Human Research Ethics Committee – Medical, University of the Witwatersrand (Clearance Certificate No: M110414) to recruit living participants to participate in the current study. The study was verbally described to potential participants and information leaflets along with informed consent forms were given to interested parties. Signing of the informed consent form confirmed their understanding and voluntary participation in the study.

Black South African females were specifically invited to participate in this study as results by Bidmos (2005) underlined the magnitude of stature underestimation in this group when using Fully's method. Black South Africans also constitute the largest population group in South Africa (Statistics South Africa, 2012) and have been shown to fall victim to crime more often than other South African population groups (Statistics South Africa, 2014). A recent study by Bernitz et al. (2015) showed that more than 70% of forensic anthropological cases comprised Black South Africans, further proof to the vulnerability of this population group to the effects of crime.

The participants included individuals from various tribal affiliations, including mainly Zulu, Xhosa, Tswana, Tsonga as well as Northern and Southern Sotho's. Research has found little intertribal variation amongst Black South Africans (De Villiers, 1968; De Beer Kaufman, 1974; Lundy, 1983, 1986) and therefore it has been suggested that these individuals be treated as a homogenous group. In addition, Franklin et al. (2008) noted that the tribal subdivisions are

disappearing. This was also evident in the current study as a number of participants self-identified as “South African” or “Black”. Moreover, in practise, forensic anthropologists will be blind to the tribal affinity of unidentified skeletal remains and as such the pooling of individuals has been suggested for research purposes (Franklin et al., 2008).

Only females between the ages of 19 and 60 years were recruited for this study. The cessation of growth is marked by the fusion of long bone epiphyses to the diaphysis and is typically completed by 18 years of age in females (Scheuer & Black, 2004). Furthermore, Trotter and Gleser (1952) found an insignificant increase in stature after the age of 18 years. An upper age limit of 60 year was selected as it has been found that the age related decrease in stature only becomes apparent after approximately 60 years of age (Cline et al., 1989).

Individuals that were claustrophobic, pregnant or breast feeding, had any skeletal abnormalities or previously broken bones as well as individuals that have suffered from growth related and/or nutritional diseases were excluded from the study. Standard Magnetic Resonance Imaging (MRI) exclusion criteria set out by the Department of Radiology, Wits Donald Gordon Medical Centre as well as those described by Shellock & Spinazzi (2008) were also adhered to.

4.2.2. Methods

4.2.2.1. Living stature

Participants were invited to complete a full body MRI scan at the Department of Radiology, Wits-Donald Gordon Medical Centre. Prior to the MRI scan the living stature (LS) of each participant was measured, as it has been found that females generally overestimate their own stature (Braziuniene et al., 2007). Living stature was measured with a stadiometer on the morning of the MRI scan so as to control for the diurnal loss of stature (Kobayashi & Togo, 1993; Sjøvold,

2000; Krishan & Vij, 2007). Living stature was measured according to standard anthropometric descriptions to the nearest 0.1 cm. Each participant was asked to change into a hospital gown and to remove their shoes and head gear. Participants were requested to stand upright with their arms at their sides, keeping the head in the Frankfurt horizontal plane (Krishan et al., 2012c).

4.2.2.2. Magnetic resonance imaging (MRI)

MRI scanograms were specifically selected as they are a non-invasive image modality that allows for the reliable and accurate assessment of skeletal remains without exposing participants to harmful ionizing radiation used by Computed Tomography (CT) scanograms or radiograms (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). The MRI scans were taken in the supine position with the head and feet of the participants supported. This was done to ensure that the participants remained in the anatomical position and also to prevent the head and feet from moving during the scan. All MRI scans were collected using a 1.5 T Phillips Entera MR Scanner (Release 3.2, Level 2, 2011-08-11 CE 0344, software version 12.1). Scans began with a T1 weighted survey with slide thicknesses of 130 mm and TR between 3000-4000. Thereafter a three station MOBI track scan was obtained from the head to the pelvis using a T2 weighted sagittal sequence at 3 mm. A three station MOBI track scan was also obtained from the pelvis to the heel using a coronal sequence. Following the full body MRI scan the MOBI track stations were fused on a MRI work station and the images transferred to a DVD. A total of 38 participants completed the full body MRI scans. Unfortunately, a number of scans were excluded due to: (i) incomplete scans and missing data; (ii) participant movement which affected the quality of the scans; and (iii) other technical errors. The exclusion of these scans resulted in a final sample size of 30 from which data was collected and subsequently analysed.

4.2.2.3. Anthropometric measurements

Skeletal measurements were collected from the MRI scanograms using the freely available image processing software, OsiriX (Rosset et al., 2004). The skeletal elements, identified by Fully (1956), that contribute directly to stature were measured according to descriptions by Raxter et al. (2006). A few measurement modifications, as suggested by Bidmos & Manger (2012), were incorporated to ensure accuracy and repeatability of the MRI measurements. Following convention, measurements were collected from the left limbs and supplemented with data from the right where needed (Moore-Jansen et al., 1994). The following measurements were collected:

1. Cranial height: This measurement was taken from basion, vertically to a point on the ectocranium directly opposite basion, as illustrated in Figure 4.1.
2. Height of C2: The height of the axis was measured on the anterior margin, between the superior tip of the odontoid process and the most inferior margin of the vertebral body (Figure 4.1).
3. Vertebral heights: the maximum vertebral body heights were measured between the most superior and inferior aspects of the body on the antero-lateral surface, excluding the pedicles and the costal facets (Figure 4.1).
4. Height of S1: The body of the first sacral segment was measured from the promontory to the most inferior aspect of the body, on the anterior surface (Figure 4.1).
5. Physiological (bicondylar) length of the femur: This measurement was taken from the most superior projecting point of the head of the femur to a line drawn below the most inferior aspects of the femoral condyles (Figure 4.2A).

6. Tibial length: The condylomalleolar length of the tibia was measured from the most inferior tip of the medial malleolus perpendicularly to a line drawn along the most superior aspect of the lateral tibial condyle (Figure 4.2B).
7. Talus-calcaneal height: Following suggestions by Dayal et al. (2008), the vertical height of the ankle was measured from the most superior point of the trochlea of the talus, perpendicular to a line that connects the most inferior aspects of the calcaneus tuberosity and the head of the 5^{th} metatarsal (plane of support) (Figure 4.3).

According to Fully's method (Fully, 1956), all the skeletal measurements described above were summed to compute the total skeletal height (TSH). Thereafter the appropriate soft tissue correction factor was added to convert the TSH to an estimate of living stature (LSF). Living stature was also estimated from the TSH using the modified anatomical methods proposed by Raxter et al. (2006) (LSR) and Bidmos & Manger (2012) (LSB).

To understand potential soft tissue correction factor differences, a general soft tissue value was calculated for the current sample, as suggested by Fully (1956), by subtracting the TSH from living stature. Bidmos & Manger (2012) found that the intervertebral discs, vertebral column curvature and the sacro-femoral height constituted the greatest portion of the soft tissue correction factor and therefore the following measurements were collected in line with suggestions by these authors:

8. Linear length of vertebral column: Measured from the most superior point of the odontoid process of the axis, linearly to the most inferior aspect of the first sacral vertebrae (Figure 4.4A).

9. Curved length of the vertebral column: Similar to the linear length of the vertebral column, this measurement was taken from the most superior point of the odontoid process of the axis to the most inferior aspect of the first sacral vertebrae; however, this measurement was taken along the anterior curvature of the vertebral column (Figure 4.4B).
10. Sacro-femoral height: This measurement was calculated in line with suggestions by Raxter et al. (2006) and Bidmos & Manger (2012) and consisted of the difference between the linear distance measured from the sacral promontory and a line connecting the superior aspects of the femoral heads (Figure 4.5), and the height of S1.

To calculate the contribution of the intervertebral discs to the soft tissue correction factor, the sum of the heights of the vertebral bodies (C2-S1) was subtracted from the linear length of the vertebral column. Similarly, the contribution of the intervertebral discs along with the curvature of the vertebral column to the soft tissue correction factor was calculated by subtracting the sum of the heights of the vertebral bodies (C2-S1) from the curved length of the vertebral column.

A number of researchers have shown that stature declines with an increase in age (Trotter & Gleser, 1951; Galloway, 1988; Cline et al., 1989; Raxter et al., 2006; Niskanen et al., 2013) and therefore the estimated statures, i.e. LSF, LSR and LSB, were adjusted to accommodate for this decline according to descriptions by Trotter & Gleser (1951) and Cline et al. (1989) and compared to living stature. The method by Trotter & Gleser (1951) was specifically selected as it has been the most commonly used age adjustment method and because it assumes an equal and linear decline in stature between the sexes. The method describe by Cline et al. (1989) on the other was chosen as it provides sex-specific, quadratic regression equations to assess stature loss with age.

4.2.2.4. Data analysis

To assess the repeatability of living stature and the anthropometric measurements, the original examiner repeated all the measurements in ten participants, approximately one month apart. The repeatability of the vertebral heights was, however, assessed by re-measuring one representative vertebra from each of the vertebral regions, i.e. C7, T10 and L5, in accordance with the work by Bidmos & Manger (2012). Repeatability was assessed using a Lin's concordance correlation coefficient (Lin, 1989).

All subsequent statistical analyses were conducted in IBM SPSS (version 20) for Windows. The data was visually assessed for outliers along with the use of the outlier labelling rules (Hoaglin et al., 1986; Hoaglin and Iglewicz, 1987). Normality was tested using a Shapiro-Wilk test (Shapiro and Wilk, 1965) and was found to be satisfied. Descriptive statistics, including the minimum, maximum, mean and standard deviation for age, physiological length of the femur, length of the tibia, TSH and living stature were calculated. A Pearson's correlation was used to assess the association between living stature and length of the femur and tibia, as well as the TSH and was visually illustrated with scatter plots. The relationship between living stature and the estimates of stature, i.e. LSF, LSR and LSB, was also assessed with a Pearson's correlation. Thereafter the accuracy of the estimated statures was tested by comparing the estimated statures with living stature, using a paired t-test.

The correlation between TSH and the general soft tissue value, calculated as the difference between living stature and the TSH, was assessed with a Pearson's correlation and visualized with a scatter plot. To further describe the soft tissue correction factor, the major contributing components, i.e. the intervertebral discs, vertebral column curvature and the sacro-femoral height

were described for Black South African females and also correlated to TSH. These values were subsequently compared to those of Black South African males, using a one sample t-test.

To assess the effects of age on stature, the difference between living stature and the estimated statures (LSF, LSR and LSB) were plotted against age. In the same way, the difference between living stature and the estimated statures adjusted for the age was also plotted against age. A Pearson's and Spearman's correlation was also used to assess the relationship between age and the difference between living stature and the adjusted and non-adjusted stature estimates, for parametric and non-parametric data respectively.

Lastly, a new soft tissue correction factor, specific for Black South African females, was calculated in line with suggestion by Raxter et al. (2006), using regression analyses. The accuracy of this equation was assessed by estimating living stature from the TSHs calculated from the MRI measurements. A range of living stature was computed by subtracting and adding one and two standard error of estimates (SEEs) to the estimated statures. The percentage of estimated statures that fell within one and two SEE was reported.



Figure 4.1. A sagittal MRI scanogram illustrating the height measurements of the cranium, axis (C2) and the vertebral bodies (C5, T10, L5 and S1); scale = 3 cm.



Figure 4.2. A coronal MRI scanogram illustrating the A) physiological length of the femur and B) tibial length measurements; scale = 5 cm.



Figure 4.3. Image illustrating the talus-calcaneus height measurement from the trochlea of the talus to the plane of support; scale = 3 cm.



Figure 4.4. Image illustrating the A) linear and B) curvilinear length measurements of the vertebral column; scale = 5 cm.

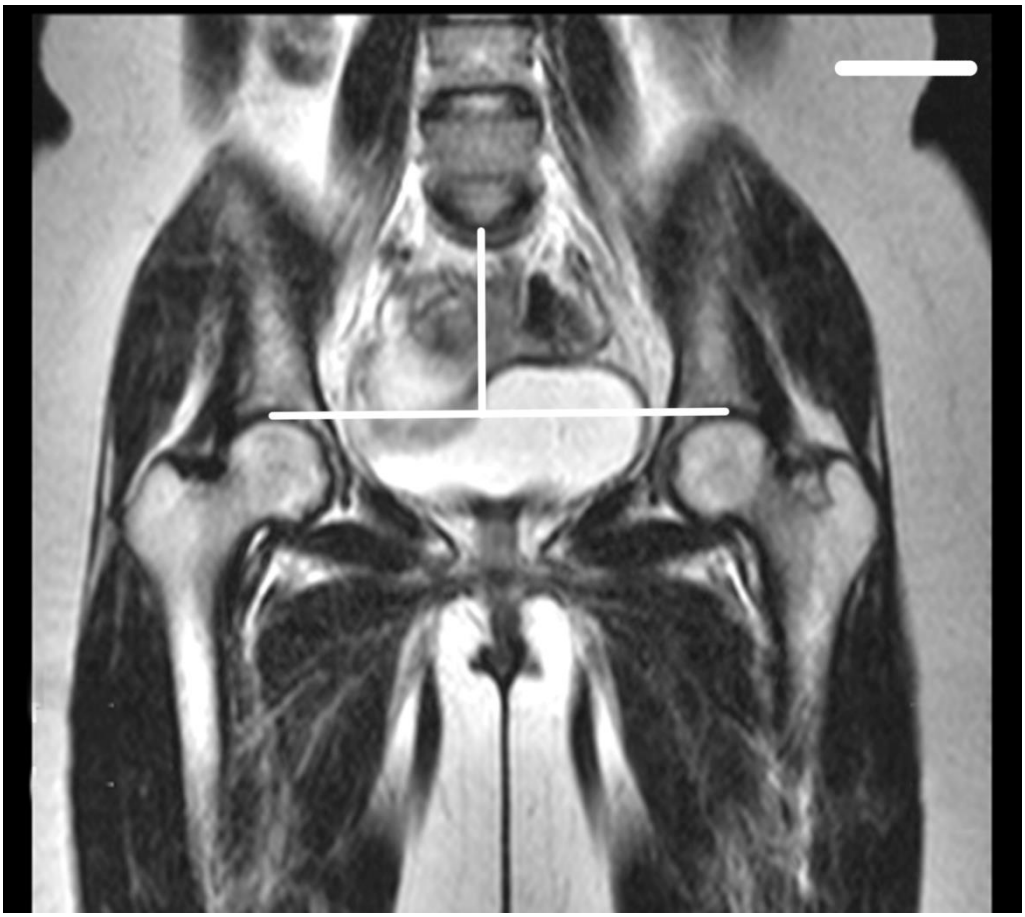


Figure 4.5. Image illustrating the measurement between the sacral promontory and a line connecting the superior aspects of the femoral heads; scale = 5 cm.

4.3. RESULTS

4.3.1. Repeatability

All measurements had Pc-value equal to or greater than 0.9, except for T10 which had a Pc-value of 0.82 (Table 4.1). This indicates that the overwhelming majority of measurements are easily reproducible.

4.3.2. Descriptive statistics

The descriptive statistics of the sample are summarized in Table 4.2. The sample included Black South African females aged between 19 and 60 years (38.0 ± 11.2), with 70% of the sample falling between 21 and 45 years of age. The TSH of the sample ranged between 127.2 and 153.1 cm (141.1 ± 5.6) and the living stature between 146.1 and 171.0 cm (159.0 ± 5.3).

The correlations between living stature and the physiological length of the femur, length of the tibia and TSH are illustrated in Figure 4.6. All the associations were statistically significant ($p < 0.0001$) and from the scatter plots it is clear that these associations are positive and linear. The strongest correlation was observed between the living stature and the TSH ($r = 0.942$), followed by the physiological length of the femur ($r = 0.879$) and lastly the length of the tibia ($r = 0.792$).

The associations between living stature and the estimated statures using Fully's method (1956) (LSF), the modified anatomical method proposed by Raxter et al. (2006) (LSR) and that by Bidmos & Manger (2012) (LSB) all showed very strong statistically significant positive correlations ($r = 0.942$, $p < 0.0001$). The paired t-test, however, indicated that Fully's method (1956) as well as the modified method proposed by Raxter et al. (2006) significantly underestimated ($p < 0.0001$) living stature by an average of 7.9 cm and 6.8 cm, respectively. The method proposed by Bidmos & Manger (2012), on the other hand, significantly overestimated

living stature ($p < 0.0001$) by an average of 7.8 cm. These estimates, i.e. LSF, LSR, LSB, also differed significantly from one another ($p < 0.0001$).

4.3.3. Soft tissue correction factor

The general soft tissue value calculated as the difference between living stature and the TSH, ranged between 13.6 and 20.9 cm (17.9 ± 1.9). Interestingly, this value showed a negligible relationship ($r = 0.024$, $p = 0.902$) to TSH and from Figure 4.7 one can see the great variability of this value. The major components of the soft tissue correction factor, as describe by Bidmos & Manger (2012), varied in its contribution. The intervertebral discs, intervertebral discs along with the curvature of the vertebral column and the sacro-femoral height amounted to an average contribution to the soft tissue correction factor of 11.6 cm, 15.1 cm and 4.1 cm, respectively. The intervertebral discs along with the curvature of the vertebral column made up the greatest component of the soft tissue correction factor and ranged between 12.9 and 17.4 cm (15.1 ± 1.2). Consistent with results for the general soft tissue value, the sacro-femoral height showed a negligible relationship ($r = -0.097$, $p = 0.615$) to TSH, while the intervertebral discs ($r = -0.323$, $p = 0.087$) and the combined value of the intervertebral discs and the curvature of the vertebral column ($r = -0.304$, $p = 0.109$) showed a moderate negative correlation to TSH. These associations were, however, not statistically significant. These soft tissue correction factor components were also compared to that reported for Black South African males and were found to be statistically significantly different ($p < 0.0001$).

4.3.4. Age effects on stature

The difference between living stature and the estimated statures, calculated using Fully's (Fully, 1956), Raxter et al.'s (2006) and Bidmos & Manger's methods (2012) were plotted against age and are illustrated in Figures 4.8A, 4.9A and 4.10A. The results show that when age is not adjusted for, there is a weak, non-significant correlation between the estimation error and age (Table 4.3). In the same way, the difference between living stature and the estimated statures adjusted for the age, using suggestions by both Trotter & Gleser (1951) and Cline et al. (1989) were also plotted against age (Figures 4.8-4.10). Results indicated that when age is adjusted for according to Trotter & Gleser's (1951) suggestions, there is no correlation with age (Table 4.3). The correlation between age and stature estimates adjusted for age according to Cline et al. (1989), showed a moderate correlation; however, these correlations were again not statistically significant (Table 4.3). Based on these results, it is concluded that age did not significantly affect the accuracy of stature estimation in the current sample.

4.3.5. Stature estimation equation

The incorrect estimation of living stature by Fully's method (1956) and the modified methods thereof, described by Raxter et al. (2006) and Bidmos & Manger (2012), highlights the need for a new soft tissue correction factor, specific for Black South African females. Thus, following suggestions by Raxter et al. (2006), regression analysis was used to compute a stature estimation equation based on the TSH. This method also incorporates a single soft tissue correction factor as opposed to the multiple soft tissue correction factors proposed by Fully (1956), as no correlation was found between the soft tissue correction factor and TSH. The computed equation for the estimation of living stature from the TSH is:

Living stature = 0.896 x TSH + 32.678 ± 1.80

This regression equation was characterized by a very strong statistically significant positive correlation ($r = 0.942$, $p < 0.0001$), emphasizing the strong relationship between the TSH and living stature. This equation also has a small SEE, indicating the high accuracy thereof.

The accuracy of the newly computed regression equation was tested by comparing living statures to a range of estimated living statures computed by adding and subtracting one and two SEE, respectively. Results indicated that 70% of the estimates of living statures fell within one and 97% within two standard errors of the estimate (SEEs).

Table 4.1. Lin's concordance correlation coefficients of reproducibility (Pc-values) for intra-observer repeatability.

Measurement	**Pc-value**
Living stature	0.98
Cranial height	0.98
Height of C2	0.96
Height of C7	0.90
Height of T10	0.82
Height of L5	0.90
Height of S1	0.91
Physiological length of femur	0.99
Tibial length	0.99
Talus-calcaneal height	0.94
Linear length of vertebral column	0.97
Curved length of the vertebral column	0.90
Sacral-femoral height	0.95

Table 4.2. Descriptive statistics, including the minimum, maximum, mean and standard deviation (SD) of the femur and tibia lengths as well as the total skeletal height (TSH) and living statures of the sample of adult females.

Participant	Age (years)	Femur length (cm)	Tibia length (cm)	TSH (cm)	Living stature (cm)
DB000002	55	45.0	37.7	143.2	160.5
DB000004	52	41.5	34.8	138.1	153.0
DB000005	30	45.9	39.1	149.4	165.5
DB000006	38	43.7	36.3	143.5	160.3
DB000007	33	46.6	39.7	146.7	165.6
DB000008	49	41.6	33.8	140.0	157.5
DB000009	42	44.8	38.7	147.7	163.1
DB000010	43	46.1	40.3	153.0	171.0
DB000011	23	41.0	33.8	132.9	152.5
DB000012	54	39.2	33.5	127.2	146.1
DB000013	28	44.3	35.9	143.0	157.6
DB000014	40	45.3	38.4	142.5	160.0
DB000015	45	44.1	37.0	146.3	165.6
DB000016	49	42.9	35.2	140.3	158.7
DB000017	60	43.6	37.5	142.6	159.6
DB000018	42	43.7	39.0	138.1	158.0
DB000019	46	41.0	34.7	136.0	154.4

Table 4.2. Descriptive statistics, including the minimum, maximum, mean and standard deviation (SD) of the femur and tibia lengths as well as the total skeletal height (TSH) and living statures of the sample of adult females (*Continued)*.

Participant	Age (years)	Femur length (cm)	Tibia length (cm)	TSH (cm)	Living stature (cm)
DB000020	37	41.8	34.3	138.2	158.8
DB000022	21	41.2	34.9	132.0	150.8
DB000023	28	40.9	34.8	137.1	155.3
DB000025	53	42.5	36.0	140.7	154.4
DB000026	25	44.4	39.7	146.9	162.1
DB000027	40	43.8	37.3	143.7	162.2
DB000029	37	43.7	37.1	142.1	163.0
DB000030	19	45.8	37.4	144.1	163.9
DB000031	25	46.3	38.7	146.3	164.0
DB000033	40	41.0	34.0	135.3	153.3
DB000035	27	44.1	35.6	142.4	161.5
DB000036	31	41.5	33.3	135.2	155.1
DB000037	29	41.8	35.3	139.5	159.7
Minimum	19	39.2	33.3	127.2	146.1
Maximum	60	46.6	40.3	153.1	171.0
Mean	38	43.3	36.5	141.1	159.1
SD	11.2	2.0	2.1	5.6	5.3

Table 4.3. Correlation coefficients (r) indicating the association between age and the differences between living stature (LS) and estimated statures (LSF, LSR and LSB) when the age related decline is factored in, following suggestion by Trotter & Gleser (1951) and Cline et al. (1981) and when it is not considered.

Variables	r-value	p-value
LS - LSF	-0.268	0.152
LS - LSF_Trotter & Gleser (1951)	0.007	0.969
LS - LSF_Cline et al. (1989)	-0.326	0.078
LS - LSR	-0.270	0.150
LS - LSR_Trotter & Gleser (1951)	0.026	0.893
LS - LSR_Cline et al. (1989)	-0.328	0.077
LS - LSB	-0.251	0.180
LS - LSB_Trotter & Gleser (1951)	-0.024	0.900
LS - LSB_Cline et al. (1989)	-0.309	0.097

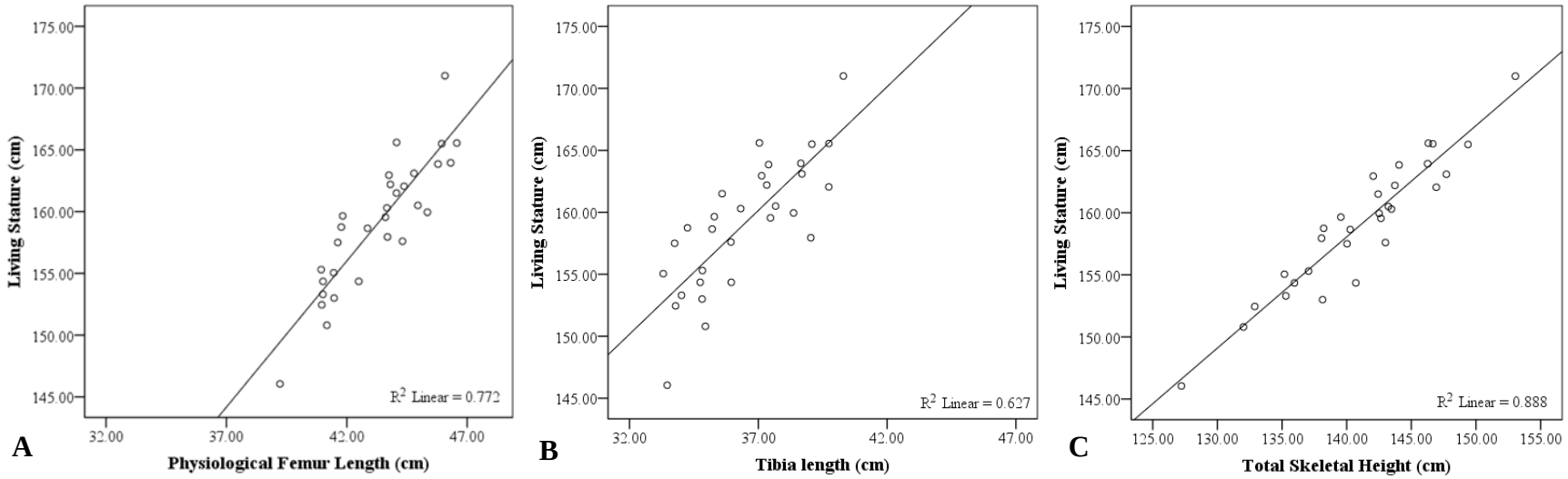


Figure 4.6. Scatter plots illustrating the association between living stature, and A) the physiological length of the femur ($r = 0.879$, $p < 0.0001$), B) tibial length ($r = 0.792$, $p < 0.0001$) and C) total skeletal height ($r = 0.942$, $p < 0.0001$).

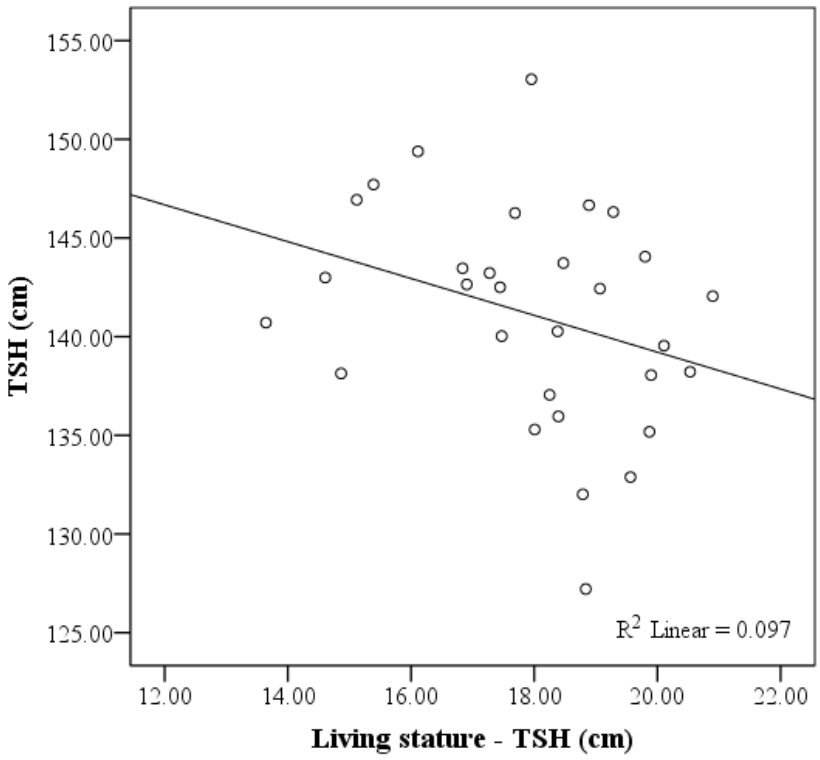


Figure 4.7. Scatter plots illustrating the lack of association between soft tissue and TSH (r = 0.024, p = 0.902).

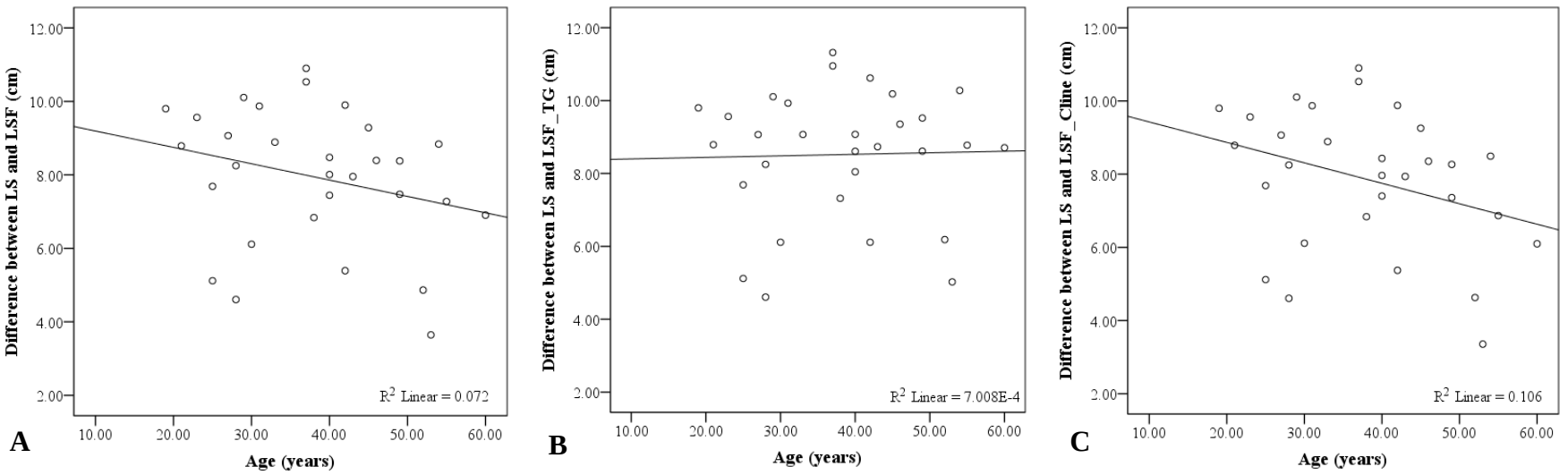


Figure 4.8. Scatter plots illustrating the association between age and the difference between living stature and A) Fully's (1956) estimated stature (LSF) without consideration for age (r = -0.268, p = 0.152), B) Fully's (1956) estimated stature (LSF_TG) adjusted for age according to suggestions by Trotter & Gleser (1951) (r = 0.007, p = 0.969) and C) Fully's (1956) estimated stature (LSF_Cline) adjusted for age according to suggestions by Cline et al. (1989) (r = -0.326, p = 0.078).

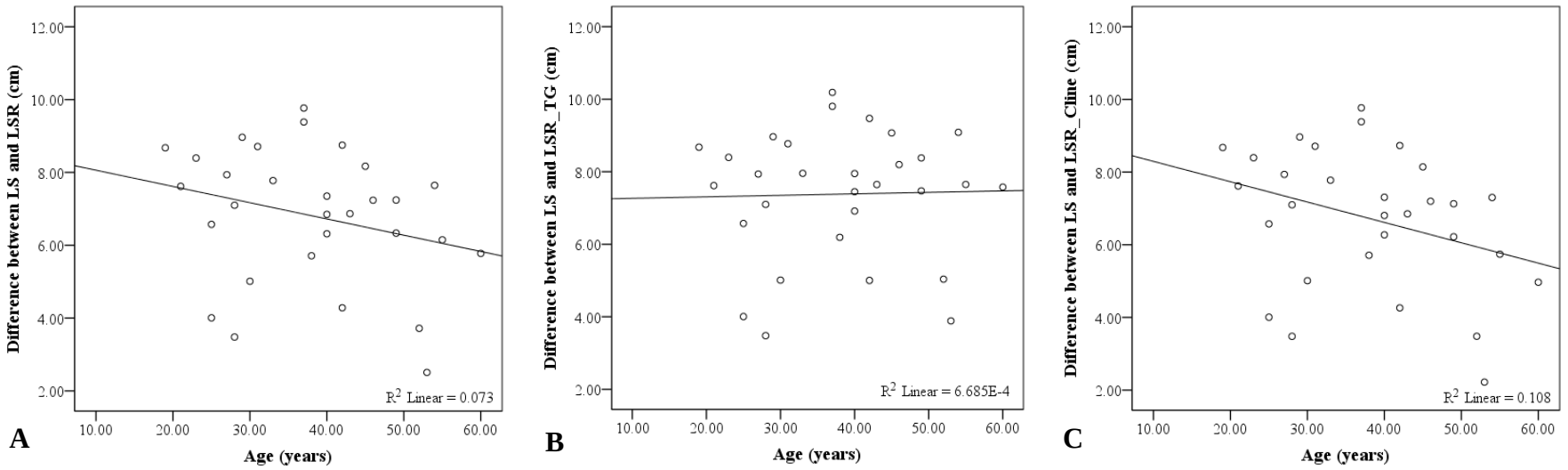


Figure 4.9. Scatter plots illustrating the association between age and the difference between living stature and A) Raxter et al.'s (2006) estimated stature (LSR) without consideration for age (r = -0.270, p = 0.150), B) Raxter et al.'s (2006) estimated stature (LSR_TG) adjusted for age according to suggestions by Trotter & Gleser (1951) (r = 0.026, p = 0.893) and C) Raxter et al.'s (2006) estimated stature (LSR_Cline) adjusted for age according to suggestions by Cline et al. (1989) (r = -0.328, p = 0.077).

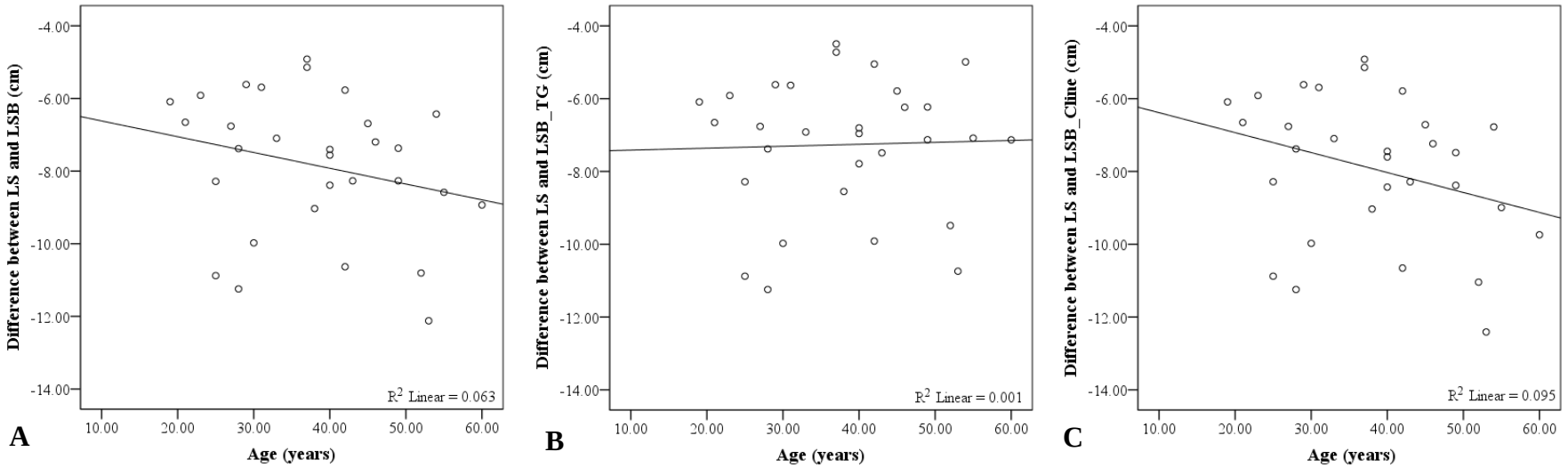


Figure 4.10. Scatter plots illustrating the association between age and the difference between living stature and A) Bidmos & Manger's (2012) estimated stature (LSB) without consideration for age (r = -0.251, p = 0.180), B) Bidmos & Manger's (2012) estimated stature (LSB_TG) adjusted for age according to suggestions by Trotter & Gleser (1951) (r = -0.024, p = 0.900) and C) Bidmos & Manger's (2012) estimated stature (LSB_Cline) adjusted for age according to suggestions by Cline et al. (1989) (r = -0.309, p = 0.097).

4.4. DISCUSSION

The anatomical method is widely accepted as the most accurate stature estimation method (Lundy, 1985, 1988; Ousley, 1995; Bidmos, 2005; Maijanen, 2009); however, the accuracy of this method has been called into question (King, 2004; Bidmos, 2005; Raxter et al., 2006; Maijanen, 2009; Bidmos & Manger, 2012). Various authors have suggested that these inaccuracies are due to the use of inappropriate soft tissue correction factors and therefore, the aim of this study was to assess the accuracy of conventional soft tissue correction factors in Black South African females and to subsequently calculate a value specific for stature estimation in this population group.

4.4.1. Magnetic Resonance imaging (MRI)

The effects of secular trends can cause some skeletal collections to no longer be representative of modern, living populations (Meadows & Jantz, 1995; Meadows Jantz & Jantz, 1999; Dirkmaat et al., 2008; Wilson et al., 2010) and therefore alternative methods are continuously being explored to study the current living population. In the current study, stature and the skeletal elements of living individuals were studied through MRI scanograms. MRI was the method of choice as it affords one the opportunity to study the skeletal system of living individuals without exposing participants to harmful ionizing radiations. Research has also shown that skeletal measurements collected from MRI scanograms are as accurate as skeletal measurements collected from CT scanograms and dry bones (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012).

The measurements collected in the current study included skeletal measurements that contribute directly to stature as listed by Fully (1956). Unfortunately some of Fully's (1956) measurement definitions were vague and various researchers have interpreted these measurements differently (Lundy, 1985, 1988; Raxter et al., 2006; Maijanen, 2009). Raxter et

al. (2006) clarified these measurements and it was suggested that the maximum bone measurements be taken where possible. Therefore, skeletal measurements collected in the current study followed suggestions stipulated by Raxter et al. (2006); however, as the measurements used in the current study were collected from MRI scanograms, a few modifications became necessary.

Cranial height is normally measured between basion, which is the most anterior point of the foramen magnum and bregma, which demarcates the interception of the sagittal and coronal sutures (Moore-Jansen et al., 1994; Raxter et al., 2006). Unfortunately bregma could not be accurately visualised on either sagittal or coronal MRI scanograms and therefore, this measurement was taken, in a sagittal scan plane, from basion, vertically to a point on the ectocranium, directly opposite the basion. This point can be considered analogous to the vertex, which is the craniometrics landmark indicating the highest point on the skull.

Measurements regarding the vertebrae have been the most controversial, with some authors measuring the height of the vertebral bodies along the anterior margin, posterior margin, anterolateral surface or in the midline (Lundy, 1985, 1988; Raxter et al., 2006; Maijanen, 2009; Bidmos & Manger, 2012). Raxter et al. (2006) found that the maximum height of the vertebral body taken on the anterolateral surface produced a more accurate estimate of living stature. Maijanen (2009), in contrast, found that stature estimates produced by the maximum midline height, taken either anterior or posterior, did not differ significantly from estimates calculated with the maximum vertebral heights as suggested by Raxter et al. (2006). Nonetheless, in keeping with suggestions by Raxter et al. (2006) the current study collected the the maximum heights between the most superior and inferior aspects of the vertebral body on the antero-lateral surface, excluding the pedicles and the costal facets.

Another measurement that has been open to interpretation is that of the height of the ankle. Adhering to the definition by Raxter et al. (2006) and Dayal et al. (2008) this

measurement was collected from the most superior aspect of the talus to the most inferior margin of the calcaneal tuberosity. Unfortunately, the feet of participants were not always at a right angle to the body and therefore, the plane of support had to be obtained by drawing a line along the most inferior aspect of the calcaneal tuberosity and the fifth metatarsal. The ankle measurement was subsequently taken from the most superior aspect of the talus perpendicular to this plane of support. This measurement modification was preferred over the suggested modification by Bidmos & Manger (2012), as their measurements fell short of the skeletal ankle height measurement by approximately 2 cm (Ruff et al., 2012).

To assess the repeatability of the MRI measurements, the original examiner repeated all the measurements in ten participants. Results from the Lin's concordance correlation coefficient (Lin, 1989) showed that all the measurements were highly repeatable, except for the vertebral body height measurements of T10. The lower repeatability observed for the vertebrae could in part be explained by the presence of vertebral osteophytes which could distort the superior and inferior margins of the vertebral bodies. Vertebral osteophytes are generally observed in individuals older than 40 year of age (İşcan & Steyn, 2013). It should, however, be noted that the *maximum* discrepancy between the original and the repeated measurements was 2 mm and falls within the error range accepted by anthropologists (Stull et al., 2013).

The living height of these individuals ranged between 146.1 and 171.1 cm (mean = 159.0 ± 5.3) and is comparable to heights reported for Black South African military females (mean = 159.6 ± 6.1) (Steyn & Smith, 2007). As such, the females from the current sample can be accepted as a representative sample of living Black South African female population. The advantage of the use of living stature includes collection of data by one observer and as such eliminates inter-observer repeatability errors, which are often a major source of error (Ousley, 1995). The use of living stature also eliminates the need for the additional adjustments such as

needed when converting cadaveric height to living height (Trotter & Gleser, 1952; Cardoso et al., 2016). The females from the current sample were significantly ($p < 0.05$) shorter compared to North American females (Fryar et al., 2012) as well as a living sample of Black South African males ($p < 0.0001$) (Bidmos and Manger, 2012). These differences attest to sex and population specificity.

4.4.2. Accuracy of soft tissue correction factors

The results obtained indicated a very strong statistically significant positive correlation between living stature and TSH, the physiological length of the femur and the tibial length. The correlation between living stature and TSH was the strongest ($r = 0.942$) and is due to the fact that the TSH comprises of the measurements of all the bones that contribute directly towards stature (Lundy, 1985; Raxter et al., 2006; Maijanen, 2009).

Similar to reports by Raxter et al. (2006) and Bidmos & Manger (2012) the estimated statures were highly correlated with living stature, but the methods described by both Fully (1956) and Raxter et al. (2006) significantly underestimated stature while that proposed by Bidmos & Manger (2012) significantly overestimated stature in Black South African females. King (2004), Bidmos (2005) and Raxter et al. (2006) also found that Fully's method (1956) underestimated stature; however, the magnitude of these underestimations (2.4 cm, 2.4 cm and 4.3 cm, respectively) was smaller than that found in the current study (7.9 cm). Conversely, the underestimation in the current study was smaller than that noted by Bidmos & Manger (2012) for both Fully's (1956) and Raxter et al.'s (2006) methods in Black South African males (15.8 cm and 14.8 cm, respectively). These differences serve to support that the present soft tissue correction factors are not appropriate for stature estimation in Black South African females.

The current study found an average soft tissue correction factor of 17.9 cm. This value is larger than that suggested by Fully (1956) (10.5 and 11.5 cm) and Raxter et al. (2006) (12.4

cm) but smaller than the soft tissue value found by Bidmos and Manger (2012) (25.9 cm). Measurements of the major components that contribute to the soft tissue correction factor, i.e. the intervertebral discs in conjunction with the curvature of the vertebral column and the sacro-femoral height were also statistically significantly smaller compared to that reported for Black South African males (Bidmos & Manger, 2012). The original soft tissue correction factor reported by Fully (1956) was calculated from a sample of European males and subsequent tests of their accuracy have shown greater inaccuracies in Black individuals, specifically females (King, 2004; Bidmos, 2005). This has led King (2004) and Bidmos (2005) to believe that the soft tissue correction factor is both sex and population specific, an inference supported by the current study. Sex- and population-specific soft tissue differences have previously been highlighted, specifically pertaining to soft tissue correction factors used for facial reconstruction (Cavanagh & Steyn, 2011).

Interestingly, the results of the current study indicated that the neither the soft tissue correction factor, nor its major components, is statically correlated to TSH ($p > 0.005$). This lack of correlation could in part also explain the underestimation of Fully's method (1956), as the magnitude of the soft tissue correction factor proposed by Fully is dependent upon the TSH. The use of a single soft tissue correction factor, as recommended by Raxter et al. (2006), in combination with regression analysis is thus more appropriate.

4.4.3. Age effects on stature

Studies have shown that stature decreases with an increase in age. This reduction has mainly been associated with the compression of the intervertebral discs and vertebral bodies along with weakening of muscles and subsequent changes in body posture (Trotter & Gleser 1951; Galloway, 1988; Cline et al., 1989; Raxter et al., 2006; Niskanen et al., 2013). Unfortunately there is no agreement regarding the age at which this decrease commences and

authors have suggested 30 (Trotter & Gleser 1951), 40 (Cline et al., 1989) and 45 (Galloway, 1988) years of age.

Results from the current study showed a weak, insignificant correlation between age and prediction errors when stature estimates were not adjusted for age. These reports are similar to those by Bidmos (2009) but differ from that of Raxter et al. (2006). The age differences of the samples utilized could explain these discrepancies. The current sample (19 – 60 years; mean 38.0), as well as the sample by Bidmos (2009) (18 – 58 years, mean 34.7), was much younger than the sample utilized by Raxter et al. (2006) (21 – 85 year; mean 54) and as noted by Cline et al. (1989) the decrease in stature between the ages of 20 and 60 is very small and are comparable to the daily diurnal variation. Cline et al. (1989) also noted that stature only starts to decrease significantly at about 60 years of age.

The results of the current study also indicated that when age-adjustments were incorporated the correlation between the estimation error and age varied between negligible and moderate albeit non-significant. Again these results were in accordance with that of Bidmos (2009). These insignificant correlations could be due to the fact that a number of factors that contribute to the age decline in stature, such as vertebral wedging, are already integrated in the anatomical method as part of the skeletal measurements collected (Raxter et al., 2006). Thus, age had no significant effect on the estimation of stature in the current sample.

4.4.4. New stature estimation equation

As the anatomical method, and modifications thereof, incorrectly estimated living stature in Black South African females a new soft tissue correction factor was calculated using regression analyses from the TSH. The regression equation was characterized by a very strong statistically significant positive correlation with living stature ($r = 0.942$). This correlation was weaker than the correlation ($r = 0.952$) reported by Raxter et al. (2006) but stronger than the

correlation (r = 0.934) reported by Bidmos & Manger (2012). The SEE for this equation was also smaller (SEE = 1.80 cm) than the SEEs reported by both Raxter et al. (2006) (SEE = 2.31 cm) and Bidmos & Manger (2012) (SEE = 1.93 cm) and attests to the high accuracy of this equation. The accuracy of this equation was also confirmed by the fact that 70% and 95% of the estimated statures fell within one and two SEEs, respectively.

4.5. CONCLUSION

The anatomical method and modifications thereof significantly under- and overestimate living stature in Black South African females. These inaccuracies have been associated with the use of universally applied soft tissue correction factors that, as illustrated in the current study, are suggested to be sex and population specificity. Based on these inaccuracies, the current study employed MRI scanograms to create a new soft tissue correction factor for stature estimation in living Black South African females. Regression analysis was used to create a stature estimation model from the TSH to estimate living stature. This model was characterized by a very strong statistically significant positive correlation to living stature and a small SEE which is indicative of the high accuracy of this method.

Numerous regression equations for the estimation of stature from various bones in Black South African females have been generated from the TSH and soft tissue correction factors proposed by Fully's (1956), due to the lack of available living statures or inaccuracies of documented cadaveric heights. The inaccuracies associated with Fully's (1956) method, as illustrated in this study, thus calls into question the precision of these regression equations and future efforts should be directed towards the re-assessment of the accuracies of these equations.

CHAPTER FIVE

Stature Estimation from the Femur and Tibia in Black South African Sub-adults

5.1. INTRODUCTION

Forensic anthropologists assist with the identification of unidentified skeletal remains by compiling an osteodemographic profile that consists mainly of age, ancestry, sex and stature estimates. Of these attributes, stature estimation can contribute to the positive identification or exclusion of an unknown individual and as such it is routinely assessed during the analysis of adult skeletal remains (İşcan & Steyn, 2013); however, when dealing with sub-adult skeletal remains emphasis is placed on the assignment of age and stature estimation is rarely attempted (Kondo et al., 2000; Lewis & Rutty, 2003; Smith, 2007; Cardoso, 2009; Sutphin & Ross, 2011). This is due to the paucity of knowledge related to stature estimation from sub-adults skeletal remains, especially for adolescents (Feldesman, 1992) and appears to be associated with the difficulties of stature estimation in sub-adults (İşcan & Steyn, 2013) where one is faced with considerable variation in growth and development between individuals and populations, changes in body proportions associated with the growth spurt and the fact that bone growth is allometric (Feldesman, 1992; Tanner, 1989; Krishan et al., 2012a). Furthermore, a general lack of standard procedures for sub-adult stature estimation could be due to age related changes often associated with juvenile stature. Additionally, the cartilaginous growth plates and bone epiphyses are rarely preserved (Baines et al., 2011) and the contribution thereof to bone length and overall stature is unknown and changes throughout growth (Lewis & Rutty, 2003).

Notwithstanding these challenges, a few stature estimation studies from foetal skeletal remains (Balthazard & Dervieux, 1921; Smith, 1939; Olivier & Pineau, 1958, 1960; Telkkä et al., 1962; Metha & Singh, 1972; Fazekas & Kósa, 1978; Krogman & İşcan, 1986) and prepubescent sub-adults have been reported (Smith, 2007; Telkkä et al., 1962; Himes et al., 1977; Banik et al., 2012; Vinitha et al., 2015). Additionally, a few studies have attempted to estimate stature from the skeletal remains of adolescents and include work by Telkkä et al. (1962), Feldesman (1992) and Ruff (2007).

The studies exploring stature estimation in adolescents are based on data collected from longitudinal growth studies, mostly conducted in the early twentieth century and utilize the diaphyseal lengths of the major long bones measured from radiographs (Feldesman, 1992; Telkkä et al., 1962; Ruff, 2007). Only the mathematical method, consisting of regression analyses and the femur:stature ratio, have been described for sub-adult stature estimation. Telkkä et al. (1962) described stature estimation in Finnish sub-adults and based on the correlations between diaphyseal long bone measurements and stature, generated stature estimation equations for individuals younger than one, individuals between one and nine years of age and for adolescents aged 10-15 years. Similarly Ruff (2007) generated age-specific stature estimation equations for sub-adults aged 1-17 years, based on radiographic data collected from the Denver Longitudinal Growth Study (Maresh & Deming, 1939; Maresh, 1955, 1970). The application of Ruff's (2007) equations is hampered by the need for age estimates to the nearest year, which is very difficult to attain when dealing with skeletal remains (Krogman & İşcan, 1986; Adams & Hermann, 2009). The errors for the stature estimation equations calculated by Ruff (2007) are comparable to that reported for adults while, standard error of estimates presented by Telkkä et al. (1962) are higher than adult errors.

Work by Feldesman (1992) explored stature estimation in sub-adults aged 8-18 years, by comparing the femur:stature ratios in sub-adults and adults. The results indicated changes in the femur:stature ratio from sub-adults to adults, with statistically significant differences noted between sub-adults aged 8-11 and adolescents between 12 and 18 years. Sex differences were also noted in the femur:stature ratio for adolescents (12 – 18 year) and the author suggested the use of a sex-specific ratio for this age group (Feldesman, 1992).

A few studies have considered the accuracies of sub-adult stature estimation equations with contradictory findings. Cardoso (2009) assessed the accuracy of the stature estimation methods described by Telkkä et al. (1962) and Feldesman (1992) on sub-adult (1 – 14 years)

skeletons, with known demographic information, from Portugal. Results indicated that both methods underestimated stature. These inaccuracies were associated with the stunted growth and proportionally shorter limbs observed in the Portuguese sample related to the low socio-economic background of these individuals (Cardoso, 2009). The accuracy of the stature estimation regressions reported by Ruff (2007) was assessed by Sciulli & Blatt (2008) as well as Sutphin and Ross (2011). Sciulli & Blatt (2008) tested the accuracy of the age-specific tibia and radius stature estimation equations on sub-adults with known demographic information, brought to the Franklin County (Ohio) Coroner. Results indicated relatively accurate stature estimates from both the maximum and diaphyseal lengths of the tibia and radius. Sutphin and Ross (2011) assessed the accuracy of the stature estimation equations on sub-adult skeletal remains from Chile's General Cemetery. Based on significant bone length and stature differences observed between Chilean and American sub-adults the authors suggested the use of these equations in prepubescent teens, but cautioned against the use in older sub-adult Chileans. These results support the cautionary note by Telkkä et al., (1962) Smith (2007), Cardoso (2009) and Baines et al. (2011) who advised against the application of sub-adult stature estimation equations on populations other than the population from which the equations were derived, due to environmental, nutritional, growth and proportional differences observed between populations (Smith, 2007; Cardoso, 2009; Baines et al., 2011; Telkkä et al., 1962).

A number of studies have also assessed the correlation between various body segments and stature, in living adolescents, including measurements of the foot (Grivas et al., 2008; Krishan et al., 2011; Krishan et al., 2012b), fingers (Krishan et al., 2012a), forearm (Song-in et al., 2013) and the head (Vinitha et al., 2015); all reporting reasonable stature estimation accuracies.

The importance of stature estimation for the positive identification of sub-adult skeletal remains has been highlighted by Imrie & Wyburn (1958), Warren et al. (1999) and Snow and

Luke (Smith, 2007) and support the need for more research. Apart from the important contribution to the positive identification of unknown individuals, estimates of stature also provide valuable information regarding growth, socio-economic status, secular change, health and nutrition of sub-adults (McCullough & McCullough, 1984; Visser, 1998; Kondo et al., 2000; Stupin & Ross, 2011; Stulp & Barrett, 2014). The need to estimate stature in sub-adults with disabilities or skeletal abnormalities is also important in clinical settings as seen in pediatric orthopedics and prosthetics (Abrahamyan et al., 2008; Adams & Hermann, 2009; Banik et al., 2012).

Adult stature estimation equations cannot be used when dealing with sub-adults remains as it greatly overestimates stature, resulting in inaccurate and unreliable results (Feldesman, 1992; Ruff, 2007; Krishan et al., 2011; Song-in et al., 2013). Therefore, due to the general lack of standards regarding the estimation of stature in sub-adults, the aim of this study was to assess the correlation between stature and lower limb bone lengths and to subsequently derive regression equations for the estimation of stature in Black South African sub-adults.

5.2. MATERIALS AND METHODS

5.2.1. Participants

Ethical approval for this study was obtained from the Human Research Ethics Committee – Medical, University of the Witwatersrand, South Africa (Clearance Certificate Number – M110414) and allowed for the recruitment of living participants. Written informed assent, prior to participation was obtained from participants who were recruited from Afrika Tikkun, Diepsloot, Johannesburg, South Africa. The parents and/or legal guardians of each participant also provided informed consent. Afrika Tikkun is a non-governmental organization that aims to empower children, youth and families through a number of community centers. Afrika Tikkun, Wings of Life Centre, Diepsloot was specifically approached for this study as

sub-adults from this community center have previously been involved in research projects (Meiring et al., 2013; Meiring et al., 2014).

Black South African sub-adults were invited and participated in this study by completing a full body Magnetic Resonance Imaging (MRI) scan. The enrollment of Black South Africans for this study was based on the fact that they do not only represent the largest population group in South Africa (79.2%) (Statistics South Africa, 2012), the skeletal remains from this population group are also more frequently encountered in forensic anthropological cases (Bernitz et al., 2015). Black South Africans consist of individuals from different subgroups including Zulu, Xhosa, Swazi and Sotho to mention a few; however, these tribal subdivisions are disappearing (Franklin et al., 2008). Previous research has also illustrated no statistically significant differences among these subgroups and as such this group was treated as a homogenous group (De Villiers, 1968; Lundy, 1983; Lundy, 1986).

Sub-adult males and females aged between 10 and 17 years were included in the study. This is in keeping with suggestions by Telkkä et al. (1962) who reported differences for stature estimation regression models in Finnish sub-adults for individuals younger than one, those between one and nine; and individuals between 10 to 15 years \. Similar reports for linear growth were also noted for infancy, childhood and adolescent periods by Maresh (1955). Children older than 17 years of age were not included as long bone epiphyses are usually fused by the age of 18, marking the cessation of growth (Scheuer & Black, 2004). Participants who have not suffered from any nutritional or growth related diseases or showed any signs of skeletal abnormalities were included. Also, participants with any previous history of fractured bones or those omitted based on standard MRI exclusion criteria, as stipulated by Shellock and Spinazzi, were also excluded from the study (Shellock & Spinazzi; 2008).

A number of sub-adults voluntarily participated in the study by successfully completing a full body MRI scan, but a few scans were excluded due to poor quality images, movement

artefacts and other technical errors. A total of 59 scans, consisting of 29 males and 30 females were included in the final analysis.

5.2.2. Data collection

The living stature of each participant was measured in an upright standing position, with a stadiometer and recorded to the nearest 0.1 cm. The examiner kept the head of each participant in the Frankfort horizontal plane by supporting the participant's chin so as to ensure that living stature was measured from the heel to the highest part of the skull (vertex) (WHO, 2008). Living stature was measured in the morning, on the day of the MRI scan, to reduce the unwanted effects of diurnal stature variation (Siklar et al., 2005).

MRI scanograms were taken in the supine position at the Department of Radiology, Wits-Donald Gordon Medical Centre, Johannesburg, South Africa using a 1.5 T Phillips Entera MR scanner (Release 3.2 Level 2, 2011-08-11, CE 0344). Scans started off with a five station T1 weighted survey with 130 mm slice thickness; TR between 3000-4000. Thereafter a MOBI track scan was obtained from the head to the pelvis using three stations and a 3 mm T2 weighted sagittal sequence. Similarly a MOBI track scan was also obtained from the pelvis to the heel in a coronal sequence. The MOBI track stations were fused on the MRI work station and measurements were collected using the freely available image processing software package OsiriX (Rosset et al., 2004).

All anthropometric measurements were collected by one observer, from the left femur and tibia. Following suggestions by Buikstra & Ubelaker (1994), measurement were taken on the right side in instances where data could not accurately be collected from the left. The diaphyseal lengths of the femur and tibia were measured, according to stipulations by Buikstra & Ubelaker (1994) and Fazekas & Kósa (1978).

1. Diaphyseal length of the femur: The maximum distance between the most proximal and distal parts of the diaphysis, parallel to the long axis of the bone (Figure 5.1A).
2. Diaphyseal length of the tibia: Similar to the diaphyseal length of the femur the diaphyseal length of the tibia was measured parallel to the long axis of the bone, between the most superior and inferior margins of the proximal and distal diaphysis (Figure 5.2A).

The diaphyseal measurements of the femur and tibia were only collected in 27 sub-adult females as the epiphyseal growth plates could not be clearly visualized in the remaining scans. Subsequently the diaphyseal measures of the femur and tibia were summed to generate an additional variable representing the skeletal length of the lower limb.

The maximum lengths of the femur and tibia were also collected, according to descriptions by Moore-Jansen et al. (1994) and Raxter et al. (2006) and also combined.

1. The maximum length of the femur was measured from the most proximal aspect of the head of the femur to the most inferiorly projecting margin of the medial condyle (Figure 5.1B).
2. The length of the tibia was measured form the most proximal aspect of the lateral condyle to the most inferior margin of the medial malleolus (Figure 5.2B).

5.2.3. Data analysis

To assess the reproducibility of the measurements, the original examiner as well as an independent observer repeated the measurements in ten scans. Intra- and inter-observer repeatability was tested using a Lin's concordance correlation coefficient (Lin, 1989). The assumption of normality was tested for stature and all measured variables, using a Shapiro-Wilk test and was found to be satisfactory (Shapiro & Wilk, 1965). Descriptive statistics,

including minimum, maximum, mean and standard deviation were calculated and a Pearson's product moment correlation was used to assess the association between living stature and the anthropometric measurements. Scatter plots were generated to visually assess these associations and linear least-squares regression equations were computed for the estimation of stature in sub-adults (Greenfield et al., 1998). Simple linear regression equations defined by $Y = a + bX$ were calculated for stature estimation, where "Y" represents stature (dependent variable), "X" the long-bone measurement (independent variable), "*a"* the intercept and "*b*" the slope (Giles & Klepinger, 1988). All analyses were conducted using the IBM SPSS (version 20) software program for Windows.

Analyses were run separately on sub-adult males and females as growth and body proportions differ significantly between sexes (Tanner, 1989; Smith & Bushcang, 2005; Nyati et al., 2006); however, analyses were also conducted on a combined sex sample due to the difficulties associated with sex estimation from sub-adult skeletal remains (Krogman & İşcan, 1986; Adams & Hermann, 2009).



Figure 5.1. Illustration of the A) diaphyseal and B) maximum length measurements of the femur, scale = 4 cm.



Figure 5.2. Illustration of the A) diaphyseal and B) maximum length measurements of the tibia, scale = 4 cm.

5.3. RESULTS

5.3.1. Repeatability

The measurements were deemed highly repeatable as all Pc-values (Table 5.1.) were greater than 0.90 (Lin, 1989). These results indicate that the measurements are reproducible using the described methodology.

5.3.2. Descriptive statistics

The sample included sub-adults aged 10-17 with an average age of 13.1 ± 2.1 years for males and 13.2 ± 2.12 years for females. The descriptive statistics of the sample and the measurements are summarised in Table 5.2. Living stature for sub-adult males ranged between 129.0 and 173.3 cm (150.4 ± 12.3cm), between 136.4 and 163.0 cm (150.7 ± 7.8) for sub-adult females and between 129.0 and 173.3 (150.6 ± 10.2) for the combined sample. Interestingly all average sub-adult female measurements were slightly greater than those of the sub-adult males, but these differences were not statistically significant ($p > 0.05$). All femur measurements were greater than those of the tibia while the maximum lengths of both bones were larger than the corresponding diaphyseal measurements.

5.3.3. Regression analyses

The correlations between the anthropometric measurements and living stature, as well as regression equations are summarized in Table 5.3. All variables showed a strong, statistically significant positive ($p < 0.0001$) correlation to living stature. These associations are illustrated in Figures 5.3-5.5. From these scatter plots it is clear that all correlations between living stature and the various anthropometric measurements were positive and linear. It is also evident that sub-adult females show greater variability compared to sub-adult males, where the distribution is closely associated with the line of best fit. The correlation coefficients were slightly greater

in sub-adult males (0.953 – 0.976) compared to females (0.875 – 0.924). For sub-adult males, the correlation coefficients for the tibia measurements were slightly greater than those of the femur, whereas the femur correlations were stronger than those of the tibia in sub-adult females. Additionally, all the diaphyseal measurements for sub-adult females showed stronger correlations compared to the maximum bone length measurements. For sub-adult males, the correlation associated with the diaphysis of the tibia was greater than the association between stature and the maximum tibia length, while the maximum length of the femur had a higher correlation than the diaphyseal length of the femur. A similar pattern was observed for the combined sample. The strongest correlation for sub-adult males was found between living stature and the combined maximum measurements for the femur and tibia ($r = 0.976$), while the combined diaphyseal measurements for the femur and tibia ($r = 0.924$) had the highest correlation for sub-adult females.

All the regression equations were characterized by small standard error of estimates (SEE) ranging between 2.71 and 3.81 cm for sub-adult males, between 3.20 and 3.85 cm for sub-adult females and between 3.16 and 3.77 cm for the combined sample. The femur SEEs were smaller in sub-adult females compared to males, while SEEs for the tibia were smaller in sub-adult males compared to females. Log transforming the data did not improve the fit of the models.

To better understand the dissimilarities observed in the correlations associated with the diaphyseal and maximum bone measurements, the size of the femur and tibia epiphyses (proximal + distal) along with the growth plates were calculated by subtracting the diaphyseal lengths from the maximum measurements (Table 5.4). The average femur and tibia epiphyses were the same for sub-adult males and females with the femur epiphyses being larger than that of the tibia. A Pearson’s product moment correlation and Spearman's rank-order correlation were calculated to assess the correlation between the epiphyses and living stature, the

diaphyseal and maximum bone measurements, for parametric and nonparametric data respectively (Table 5.5). Results indicated no correlations associated with the tibia epiphysis ($p > 0.05$), however, the femur epiphyses was significantly correlated with living stature, diaphyseal length and maximum length of the femur in sub-adult males ($p < 0.05$), but not for females.

Table 5.1. Lin's concordance correlation coefficients of reproducibility for intra- and inter-observer repeatability.

Measurement	Intra-repeatability	Inter-repeatability
Femur diaphysis	0.993	0.995
Maximum femur	0.996	0.995
Tibia diaphysis	0.997	0.986
Maximum tibia	0.995	0.902

Table 5.2. Descriptive statistics, including the minimum (min), maximum (max), mean and standard deviation (SD), for the sample and anthropometric measurements (cm).

	Males					Females					Combined				
Measurements	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**
Living stature	29	129.0	173.3	150.4	12.3	30	136.4	163.0	150.7	7.8	59	129.0	173.3	150.6	10.2
Femur															
Diaphyseal length	29	30.9	44.0	37.6	3.5	27	32.7	41.9	38.1	2.6	56	30.9	44.0	37.8	3.1
Maximum length	29	34.3	48.7	41.5	3.8	30	36.2	45.9	41.8	2.6	59	34.3	48.7	41.7	3.3
Tibia															
Diaphyseal length	29	25.1	37.7	31.4	3.3	27	28.0	36.2	32.3	2.4	56	25.1	37.7	31.8	2.9
Maximum length	29	27.7	40.7	34.3	3.4	30	30.9	39.2	35.0	2.5	59	27.7	40.7	34.7	2.9
Femur + tibia															
Diaphyseal length	29	56.1	81.7	69.0	6.7	27	60.7	77.4	70.4	4.9	56	56.1	81.7	69.7	5.9
Maximum length	29	62.0	89.4	75.8	7.2	30	67.2	84.7	76.8	5.0	59	62.0	89.4	76.3	6.1

Table 5.3. Correlation coefficients and regression equations[b] for the estimation of stature in black South African sub-adult males, females and a combined sex sample.

	Males					Females					Combined				
Measurements	**r**	**r^2**	**Slope**	**Intercept**	**SEE**	**r**	**r^2**	**Slope**	**Intercept**	**SEE**	**r**	**r^2**	**Slope**	**Intercept**	**SEE**
Femur															
Diaphyseal length	0.953	0.907	3.349	24.603	3.81	0.914	0.836	2.908	39.844	3.40	0.937	0.878	3.180	30.265	3.69
Maximum length	0.965	0.931	3.087	22.391	3.29	0.912	0.831	2.702	37.626	3.26	0.947	0.897	2.954	27.489	3.30
Tibia															
Diaphyseal length	0.975	0.950	3.632	36.343	2.80	0.903	0.815	3.041	52.464	3.60	0.942	0.887	3.362	43.510	3.53
Maximum length	0.966	0.934	3.510	29.935	3.23	0.875	0.765	2.768	53.862	3.85	0.930	0.865	3.210	39.278	3.77
Femur + tibia															
Diaphyseal length	0.974	0.948	1.781	27.581	2.85	0.924	0.854	1.538	42.406	3.20	0.952	0.906	1.678	33.661	3.23
Maximum length	0.976	0.953	1.679	23.138	2.71	0.909	0.825	1.414	42.097	3.32	0.952	0.905	1.580	29.969	3.16

[b]*Estimated living stature = intercept + slope x variable ±SEE*

Table 5.4. Descriptive statistics, including the minimum (min), maximum (max), mean and standard deviation (SD), for the femur and tibia epiphyses (cm).

	Males					Females					Combined				
Epiphyses	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**
Femur	29	3.1	4.9	3.9	0.6	27	3.2	4.7	3.9	0.4	56	3.1	4.9	3.9	0.5
Tibia	29	2.1	4.1	2.9	0.5	27	2.3	4.1	2.9	0.4	56	2.1	4.1	2.9	0.5

Table 5.5. Correlation coefficients for associations between the epiphyseal length, living stature as well as the diaphyseal and maximum bone length measurements (cm).

	Males						Females						Combined					
Epiphyseal length	**Stature**		**Diaphyseal length**		**Maximum length**		**Stature**		**Diaphyseal length**		**Maximum length**		**Stature**		**Diaphyseal length**		**Maximum length**	
	p	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**
Femur	<0.000*	0.652	0.002*	0.545	<0.000*	0.647	0.055	0.374	0.404	0.167	0.112	0.313	<0.000*	0.567	0.001*	0.419	<0.000*	0.539
Tibia	0.581	0.107	0.625	0.095	0.681	0.080	0.560	0.117	0.868	0.034	0.295	0.209	0.420	0.110	0.673	0.058	0.183	0.180

**Correlation significant at 0.01 level (2-tailed)*

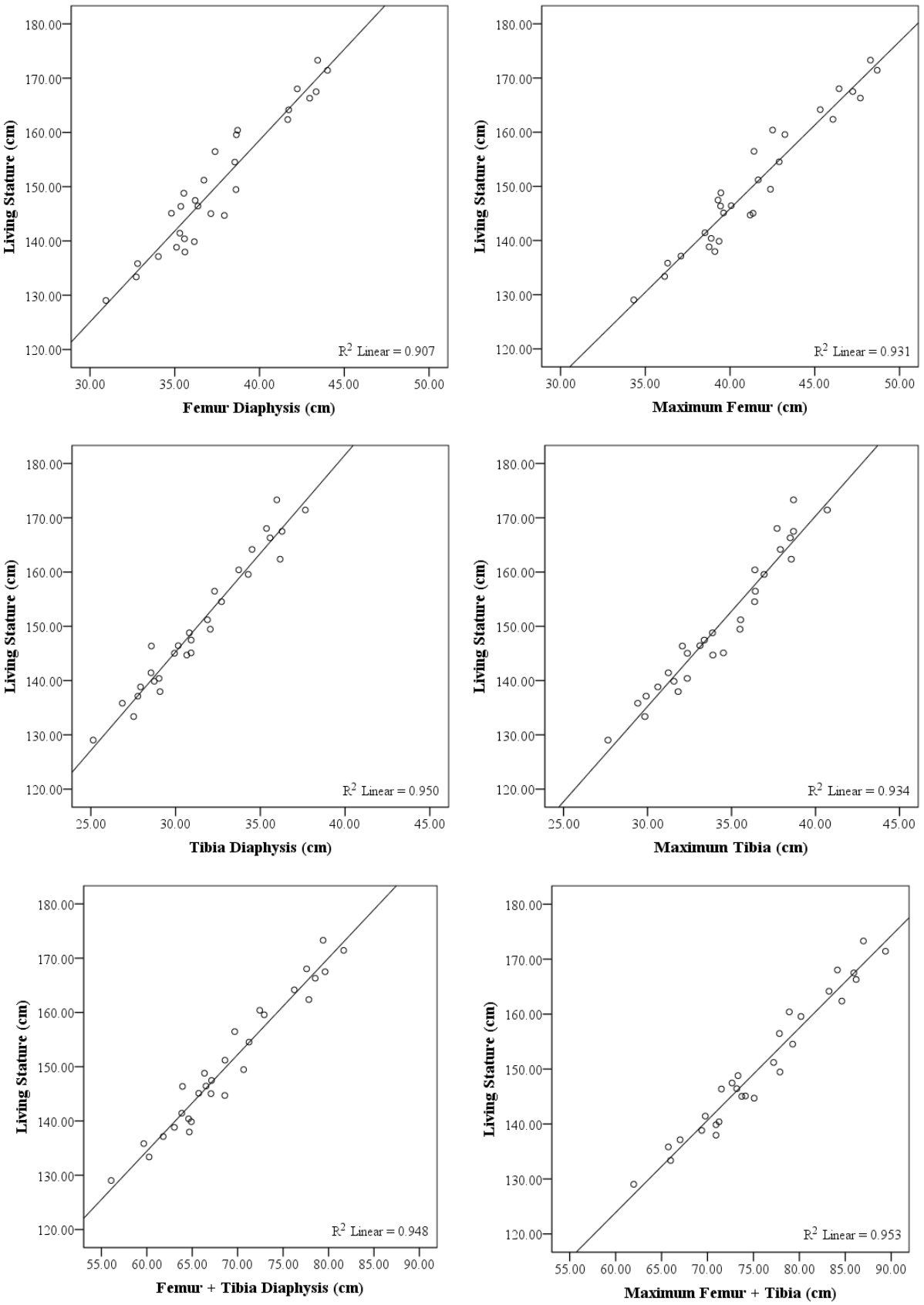


Figure 5.3. Scatter plots illustrating the association between living stature, and the diaphyseal and maximum lengths of the femur and tibia in sub-adult males.

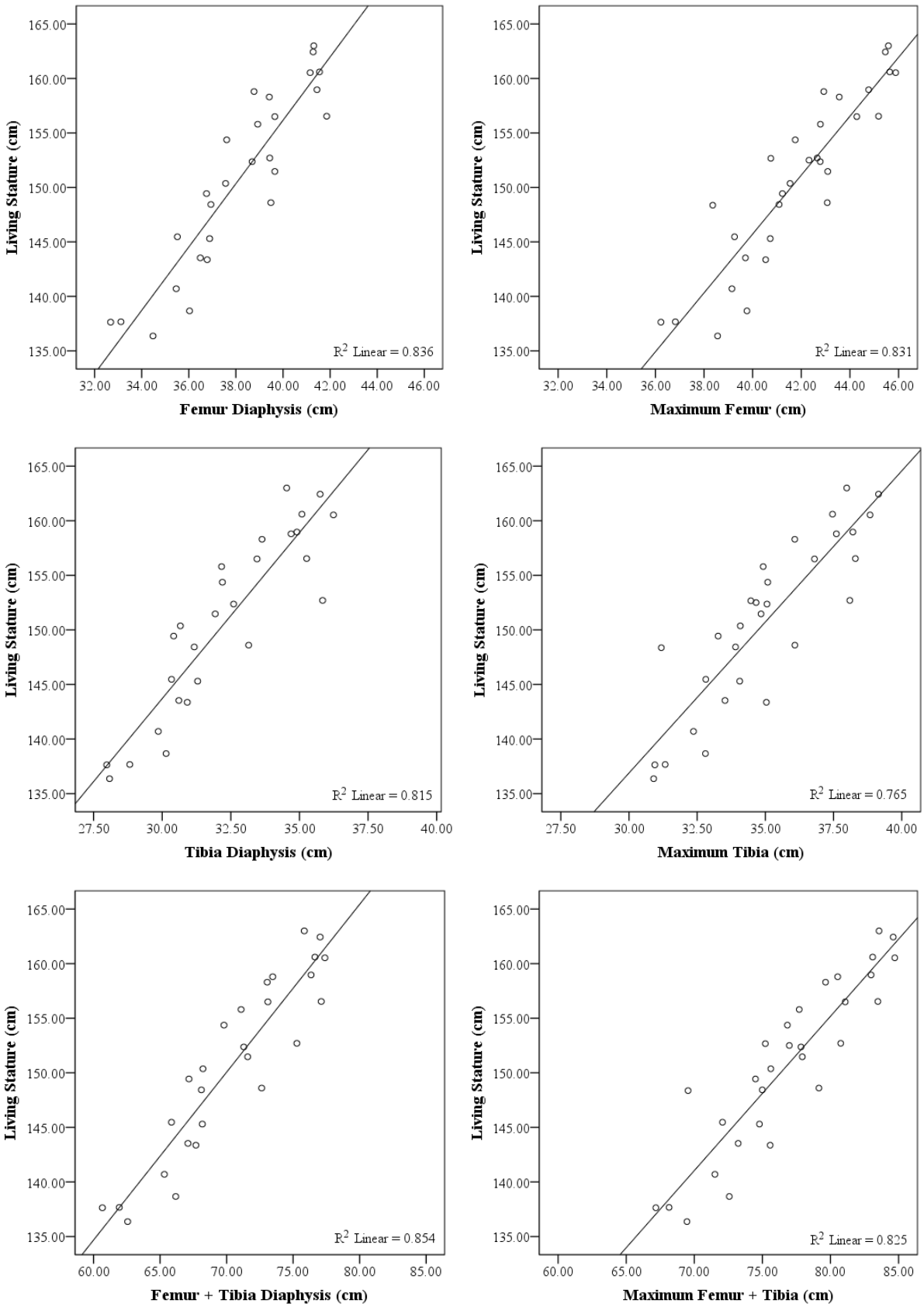


Figure 5.4. Scatter plots illustrating the association between living stature, and the diaphyseal and maximum lengths of the femur and tibia in sub-adult females.

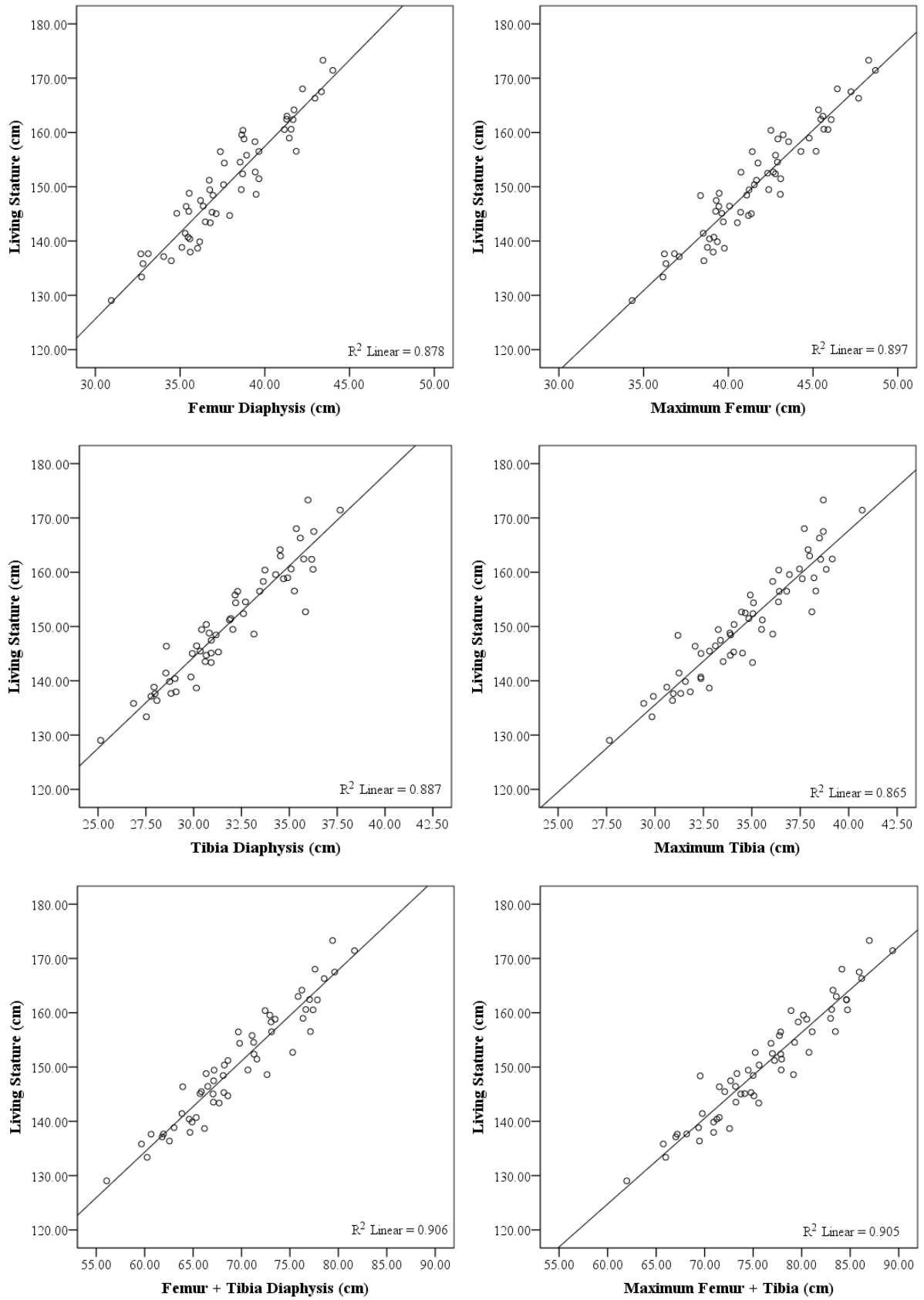


Figure 5.5. Scatter plots illustrating the association between living stature, and the diaphyseal and maximum lengths of the femur and tibia in a combined sex sample of sub-adults.

5.4. DISCUSSION

Research on sub-adult remains is hampered by the lack of large, modern, sub-adult skeletal collections with known demographic information, especially stature (Telkkä et al., 1962; Lewis & Rutty, 2003; İşcan & Steyn, 2013). As such most studies involved in sub-adult stature estimation rely on data collected from growth related studies (Feldesman, 1992; Visser, 1998; Ruff, 2007; Smith, 2007) utilizing radiographic imaging (Telkkä et al., 1962; Himes et al., 1977; Feldesman, 1992; Visser, 1998; Ruff, 2007; Smith, 2007; Abrahamyan et al., 2008). Limitations of radiological studies involve image distortions that lead to measurement errors as well as the exposure of participants to harmful radiation (Telkkä et al., 1962; Leitzes et al., 2005; Ruff, 2007; Doyle & Winsor, 2011; Rathnayaka et al., 2012). Presented here is an alternative approach to study sub-adult skeletal remains from living participants. MRI is a safe, non-invasive imaging modality that can be used to assess the musculoskeletal system without exposing participants to harmful radiation (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). Although MRI is not routinely used for bone analyses, due the lack of a bone MR signal, it can still be accurately examined based on the MR signal produced by the surrounding tissue (Rathnayaka et al., 2012). Measurements collected from MRI's are comparable to data collected from CT scanograms and dry bones and as such MRI measurements are considered accurate and reliable (Leitzes et al., 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). MRI scanograms also afford the opportunity to study skeletal remains of living individuals, essentially excluding the unwanted effects of secular trends that often affect skeletal collections.

This study benefitted from the collection of living stature of participants as opposed to the use of cadaveric height, which has been found to overestimate living stature (Cardoso, 2009). The Black South African sub-adults from the current study were taller than Black South African sub-adults measured as part of the Pretoria National Nutrition Survey (PNNS) between

1963 and 1965 (Hawley et al., 2009). This increase in stature is in line with the general secular increase in height seen in various populations and is thought to be related to the overall improvement in health and nutrition (Tanner, 1989; Bogin & Rios, 2003; Stulp & Barrett, 2014). The current sample was, however, slightly shorter than Black South African sub-adults measured in the Birth-To-Twenty (Bt20) programme between 1999 and 2001 (Hawley et al., 2009). This difference in mean living stature is most likely due to the small sample size available for direct age group comparisons in the current study. Living stature of sub-adults from the current sample was comparable to that of Zimbabwean sub-adults (Olivieri et al., 2008), but slightly shorter compared to White South Africa (Hawley et al., 2009), European (Abrahamyan et al., 2008) and North American (Maresh, 1955; Eveleth et al., 1979; Fryar et al., 2012) sub-adults of similar ages. The Black South African sub-adults were, however, taller than Mayan (McCullough & McCullough, 1984), North Indian (Krishan et al., 2011, 2012b) and Mexican American (Malina et al., 1987) sub-adults, with the exception of some age groups. This again appears to be linked to the small age specific sample available for direct comparisons with other studies.

Direct comparisons of the femur and tibia measurements to other studies could not be made due to non-standard anthropometric measurements (Telkkä et al., 1962; Abrahamyan et al., 2008) radiographic measurement enlargements (Maresh & Deming, 1939, Maresh, 1955; Himes et al., 1977; Visser, 1998; Smith, 2007) and different age groups studied (Himes et al., 1977; Smith, 2007; Banik et al., 2012; Ibegbu et al., 2015; Vinitha et al., 2015). Diaphyseal lengths of the femur and tibia for Black South African sub-adults were larger than those reported for Inuit sub-adults (Y'Edynak, 1976), while both the diaphyseal and maximum length measurements of the femur and tibia were smaller compared to that reported for North American sub-adults (Maresh, 1955; Ginhart, 1973; Smith & Buschang, 2005; Sciulli & Blatt, 2008). Interestingly, Black South African sub-adult females had tibia measurements

comparable to that of Black Americans, whilst being somewhat larger than those reported for White American sub-adult females (Sciulli & Blatt, 2008).

A number of reasons can be put forward to explain the differences observed in height and bone measurements between the populations. The larger measurements observed for the Black South African sub-adults compared to some populations could in part be due secular changes which often result in the increase in height and skeletal measurements due to improvements in health, nutrition, medical care and the environment (Meadow Jantz & Jantz, 1970; Tanner, 1989; Bogin & Rios, 2003; Stulp & Barrett, 2014). Another source of variation is related to differences observed in growth, including the onset, duration and the tempo thereof (Meadow Jantz & Jantz, 1970; Tanner, 1989; Cole et al., 2015). Different individuals and populations also grow at different rates which are mainly due to genetic and environmental influences. In addition, different axial and appendicular bones grow at different rates producing different body proportions (Malina et al., 1987; Tanner, 1989; Bogin & Rios, 2003; Nyati et al., 2006). Socio-economic status can also introduce variation in growth and as such differences between populations. Research has shown that individuals from lower socio-economic strata, such as Black South Africans sub-adults representing individuals from developing countries, often show impaired and or delayed growth and bone maturation compared to individuals from higher socio-economic segments (Stulp & Barrett, 2014).

Black South African sub-adult females were slightly taller than sub-adult males and had larger diaphyseal and maximum length measurements until approximately 15 years of age, when sub-adult males surpassed the sub-adult females. Similar findings were reported by Grivas et al. (2008) and Smith and Buschang (2005). This can be ascribed to the fact that sub-adult females mature earlier than sub-adult males, by entering their adolescent growth spurt approximately two years prior to sub-adult males (Feldesman, 1992; Smith & Buschang, 2005; Cole et al., 2015). Therefore, sub-adult females reach their adult height earlier than males

(Tanner, 1989) and have somewhat longer bone lengths (Smith & Buschang, 2005). Sex differences are also brought about by growth related variations as described above. The differences illustrated between the sexes and populations thus support the need for sex and population specific stature estimation equations.

A few sub-adult stature estimation studies were available for comparisons. The strong linear correlations observed in the current study were similar to correlations reported for long bones amongst Thai (Song-in, 2013), Mexican (Banik et al., 2012) and French (Abrahamyan et al., 2008) sub-adults, but greater than that reported for the foot (Grivas et al., 2008; Krishan et al., 2011, 2012b), fingers (Krishan et al., 2012a), hand (Ibegbu et al., 2015) and head (Vinitha et al., 2015) measurements. The strong correlations between long bones and stature, specifically lower limb bones, are due to the fact that these bones contribute directly to the overall height of the body (Nyati et al., 2006; Ruff, 2007). Likewise the SEE for the sub-adult stature estimation equations were comparable to that reported for long bones (Telkkä et al., 1962; Himes et al., 1977; Ruff, 2007; Smith, 2007; Song-in, 2013) and considerably smaller than errors recorded for the foot (Grivas et al., 2008, Krishan et al., 2011, 2012b) and finger (Krishan et al., 2012a) stature estimation models. Congruent with reports by Grivas et al. (2008), Krishan et al. (2012a), Banik et al. (2012) and Ibegbu et al. (2015) males showed stronger correlations compared to females. Furthermore, a stronger correlation along with smaller standard error of estimates were observed between living stature and the femur in sub-adult females, while the tibia had a stronger correlations and smaller SEE for sub-adult males. Visser (1998) and Ruff (2007) also found the tibia to produce more accurate estimates than the femur in younger age groups. This is thought to be related to fact that the tibia increases in length faster than the femur (Smith & Buschang, 2005) and as such the growth of the tibia is completed before that of the femur (Scheuer & Black, 2004). The fact that the femur produced more accurate results in sub-adult females is due to the earlier onset of the growth spurt

whereby females reach their adult size prior to sub-adult males (Tanner, 1989; Scheuer & Black, 2004; Smith & Buschang, 2005).

The correlations observed in the current study exceed that reported for Black South African adults (Lundy & Feldesman, 1987) and White South African adult males (Dayal et al., 2008). The sub-adult female correlations are, however, somewhat smaller than that reported for White South African adult females (Dayal et al., 2008). Similar to reports by Telkkä et al. (1962) the SEE for the sub-adult stature estimation equations is slightly larger than those reported for adults (Lundy & Feldesman, 1987; Dayal et al., 2008). The larger SEE in sub-adults can be explained by the ongoing growth spurt, which results in a continuous change in the correlation of parameters (tanner, 1989; Smith & Buschang, 2005). As expected, the combined measures of the femur and tibia produced stronger correlations and smaller stature estimation errors, congruent with findings by Ruff (2007) and Smith (2007) as it contributes to a greater portion of overall height (Ruff, 2007).

Sub-adult stature estimation in the past has also been hindered by the lack of information pertaining to the contribution of the cartilaginous growth plates and bone epiphyses to bone length as well as living stature (Krogman & İşcan, 1986; Lewis & Rutty, 2003). Noteworthy, the diaphyseal and maximum length measurements of both bones in the current study produced stature estimation equations with comparable accuracies. This is related to the lack of statistically significant correlations between bone epiphyses and living stature, maximum and diaphyseal bone lengths as illustrated in the current study. These results are consistent with reports by Seitz (1923) who also found no correlation between the humerus and tibia epiphyses and bone length. The femur epiphyses in the current study did show significant correlations to living stature, diaphyseal and maximum length measurements in sub-adult males and the combined sex groups, and might explain why the maximum length of the femur provided slightly better results than the diaphyseal length of the femur.

5.5. CONCLUSION

This study is limited in its conclusions due to the small sample size available for analysis. This is partly due to the increased scanning time and costs involved in acquiring MRI scanograms for analysis (Leitzes et al., 2005; Rathnayaka et al., 2012). The small sample also necessitated pooling age groups that have shown to produce lower stature estimation accuracies (Ruff, 2007; Smith, 2007; Krishan et al., 2012b). Notwithstanding these limitations, the current study illustrated sex and population differences that confirm the need for sex and population specific sub-adult stature estimation equations. Results indicated strong statistically significant correlations between living stature and the diaphyseal and maximum length measurements of the femur and tibia of Black South African adolescents, along with small standard error of estimates for stature estimation models. These correlations and error rates are comparable to stature estimation regression equations reported for Black South African adults (Cole et al., 2015). Therefore, the anthropometric variables described in this study can be considered good estimators of sub-adult stature that will contribute valuable information to the biological profile of unidentified sub-adult skeletal remains.

Future studies will benefit from increased sample sizes, exploring the correlation between various bone measurements and living stature for age and sex specific groupings. Efforts in understanding the size, growth and correlations of bone epiphyses will also greatly benefit future sub-adult stature estimation investigations.

CHAPTER SIX

Assessing the Use of the Anatomical Method for the Estimation of Sub-adult Stature in Black South Africans

6.1. INTRODUCTION

Two methods are available for the estimation of stature from skeletal remains: the mathematical method, which includes stature:bone ratios and regression analyses, and the anatomical method (Lundy, 1985; Moore & Ross, 2013). The mathematical method is considered to be the most commonly used method as it allows for the estimation of stature from a single bone or a combination of bones (Tatarek et al., 2005). The stature estimation equations generated using the mathematical are, however, sex and population specific and should not be applied to populations other than the population from which it was derived (Trotter & Gleser, 1958; Sjøvold, 2000; Moore & Ross, 2013).

The anatomical method, also known as Fully's method (Fully, 1956), is described as the most accurate stature estimation method, benefitting from the inclusion of all the skeletal elements that contribute directly to stature (Lundy, 1985; Ousley, 1995; Raxter et al., 2006). Measurements collected from these bones are summed to generate the total skeletal height to which a soft tissue correction factor is added to produce an estimate of living stature (Fully, 1956). The soft tissue correction factor was initially believed to be independent of sex and population affinity (Lundy, 1985; Raxter et al., 2006), however, a number of recent publications have questioned this (King, 2004; Bidmos, 2005; Bidmos & Manger, 2012).

The mathematical and anatomical methods are well defined for stature estimation relating to adult skeletal remains; however, stature estimation from sub-adult skeletal remains is rarely attempted (Kondo et al., 2000; Lewis & Rutty, 2003). This is due to a general lack of available literature and standards, related to the shortage of sufficiently large sub-adult skeletal collections with known demographics, available for research (Sundick, 1977; Lewis & Rutty, 2003). Research on sub-adult stature estimation is further encumbered by individual and population differences in growth and development as well as the allometric growth of bones,

which causes considerable change in body proportions throughout life (Eveleth & Tanner, 1976; Tanner, 1989; Bogin & Rios, 2003; Nyati et al., 2006; Cole et al., 2015).

To date only the mathematical method has been explored for stature estimation from sub-adult skeletal remains. This includes work by Feldesman (1992) on the femur:stature ratio as well as a number of studies that have computed regression equations for sub-adult stature estimation from several skeletal remains, including the length of the second metacarpal (Himes et al., 1977) and lengths of various upper and lower limb long bones (Palkama et al., 1962 Telkkä et al., 1962; Virtama et al., 1962; Metha & Singh, 1972, Fazekas & Kósa, 1978; Ruff, 2007; Smith, 2007; Brits et al., 2016).

The anatomical method has not yet been described for stature estimation in sub-adults; however, a method comparable to that described by Dwight (1894) is available. Kondo et al. (2000) aimed to estimate stature from the well preserved skeletal remains of an immature Neanderthal skeleton. The authors reconstructed and re-articulated the skeleton using casts derived from the original skeletal remains, while missing bones and/or bone sections were restored with paraffin wax. The curvature of the vertebral column as well as the intervertebral distances, joint cartilage thicknesses and the missing talus/calcaneus complex were established from photographs and radiographs of modern sub-adults. Stature was measured directly from the reconstructed skeleton and adjusted to incorporate the shrinkage associated with the use of casts. Finally, a soft tissue correction factor of 10 mm, representing the scalp and sole thicknesses was also added. The authors asserted that this is an accurate method for sub-adult stature estimation as it eliminates proportional differences observed between different body parts and between various populations (Kondo et al., 2000). The veracity of this assertion based on a method developed for a single sub adult Neanderthal requires further investigation in which a larger sample size is utilized. Consequently, this observation should be treated with caution.

Due to the scarcity of information available on sub-adult stature estimation, the aim of this study was to assess the use of the anatomical method for stature estimation in Black South African sub-adults.

6.2. MATERIALS AND METHODS

6.2.1. Participants

Due to the paucity of sub-adult skeletal collections available for research (Sundick, 1977; Lewis & Rutty, 2003) living participants were recruited to partake in this study by completing a full body Magnetic Resonance Imaging (MRI) scan. MRI scans were specifically chosen for this study as it affords the opportunity to study the internal structures of living individuals, including the musculoskeletal system, without exposing participants to harmful ionizing radiation (Ecklund, 2002; Leitzes, 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012). Additionally, skeletal measurements collected from MRI scans have been found to be as accurate and reliable as measurements collected from CT scans and dry bones (Leitzes, 2005; Doyle & Winsor, 2011; Rathnayaka et al., 2012).

Black South African sub-adult males and females from the greater Johannesburg area were recruited to voluntarily participate in this study. Informed assent and consent was obtained from the participants as well as their parents or legal guardians, prior to participation. Black South Africans were of specific interest to this study as they constitute the largest population group in South Africa (Statistics South Africa, 2012). The skeletal remains from this population also makeup a greater number of forensic anthropological cases compared to other South African groups (Bernitz et al., 2015). The participants represented various South African tribes, including Venda, Ndebele, Xhosa, Sepedi, Zulu, Sotho, Tswana and Tsonga; however, a large number of participants self-identified as “South African” or “Black” and as

such no tribal distinctions were made. This is in line with reports by Franklin et al. (2008) who found that tribal sub-classifications in South Africa are disappearing.

Only sub-adults aged between 10 and 17 years were included in the study. This age range was of specific interest due to the general lack of reach related to stature estimation in adolescents (Feldesman, 1992). Additionally, the lower age limit was selected to ensure an increased sample size, as research has shown that more than 50% of participants aged 5 years and older, successfully completed MRI scans (Byars et al., 2002; Malisza et al., 2010). It has also been found that sedation is not required for MRI scans for individuals 6 years and older (Leitzes et al., 2005). An upper age limit of 17 years was set, as fusion between long bone diaphyses and epiphyses usually occur around the age of 18, signifying the end of long bone growth (Scheuer & Black, 2004).

Standard MRI exclusion criteria were adhered to and participants with any metal implants or devices or pregnant female participants were excluded from the study (Shellock & Spinazzi, 2008). Participants who have suffered from nutritional deficiencies or growth related diseases, skeletal abnormalities or those who have reported broken or fractured bones were also excluded.

Ethical clearance was granted by the Human Research Ethics Committee (Medical), University of the Witwatersrand, South Africa (Clearance Certificate Number – M110414).

6.2.2. Methods

6.2.2.1. Magnetic Resonance Images (MRI) Scans

A 1.5-T Phillips Entera MR Scanner, housed at the Department of Radiology, Wits-Donald Gordon Medical Centre was used to collect MRI scans. The examinations were carried out with participants in the supine position and started with a sagittal T1-weighted survey scan (TR between 3000-4000, 130 mm slice thickness). This was followed by 3 mm T2-weighted MOBI

track scans using three stations. T2-weighted scans were preferred as it produces images that allowed for easier differentiation between the long bone epiphyses and the growth plates (Ecklund, 2002). A sagittal sequence from the head to the pelvis was taken, followed by a coronal sequence from the pelvis to the heel. The sagittal and coronal MOBI track stations were fused together on a workstation and the images saved to a digital versatile disc (DVD).

A total of 67 volunteers participated in the study; however, a number of participants did not successfully complete the MRI scan. The sample size was further reduced by the exclusion of poor quality scans and scans affected by movement and other technical errors. The final sample available for analyses consisted of 53 sub-adults, including 24 males and 29 females.

6.2.2.2. Anthropometry

Living stature was measured in the morning, prior to the MRI scan, so as to decrease the effect of diurnal variation of stature (Siklar et al., 2005). Living stature was collected according to stipulations by the World Health Organization with the participant standing upright and the head in the Frankfort horizontal plane. Measurements of height were collected with a stadiometer and recorded to the nearest 0.1 cm (WHO, 2008).

Anthropometric data was collected from the MRI images using the image processing software OsiriX (Rosset et al., 2004). As per convention, long bone measurements were taken on the left side but were supplemented with data from the right where required (Buikstra & Ubelaker, 1994). All the skeletal elements that contribute directly to stature were measured as described by Moore-Jansen et al. (1994) and Raxter et al. (2006), with some modifications as suggested by Bidmos & Manger (2012) for the collection of skeletal measurements from MRI scans. A number of sub-adult specific skeletal measurements were also collected, following descriptions by Fazekas & Kósa (1978) and Buikstra & Ubelaker (1994). These measurements were included as the nature of immature remains varies depending on age-at-death and as such

will affect the method to be used during skeletal analysis along with the accuracies thereof. Measurements included the following:

1. Cranial height: The height of the cranium or the basi-bregmatic height was measured from the basion vertically, to an opposite point on the skull (Figure 6.1). This was done as bregma could not be accurately identified from the MRI scans.
2. Height of the axis: The vertical height of the axis was measured on the anterior margin, from the most superior tip of the odontoid process to the most inferior aspect of the body (Figure 6.1).
3. Maximum vertebral heights of C3 to L5: The maximum height of the vertebral bodies was measured on the anterolateral surfaces, applying caution not to include the costal facets (thoracic vertebra) and/or the pedicles (Figure 6.1).
4. Height of the first sacral segment: The median maximum height of the first sacral vertebra was measured on the pelvis surface, between the promontory and the inferior aspect of the sacral body (Figure 6.1).
5. Diaphyseal length of the femur: The maximum distance, parallel to the long axis of the bone, between the proximal and distal epiphyses (Figure 6.2A) was measured.
6. Physiological length of the femur: This measurement was collected from the most superior point of the head of the femur to a point on a line connecting the inferior margins of the femoral condyles (Figure 6.2B).
7. Maximum length of the femur: This measurement was taken between the most superior parts of the femoral head the most inferior projection of the medial condyle (Figure 6.2C).
8. Diaphyseal length of the tibia: The maximum diaphyseal length of the tibia was measured similar to that of the femur, parallel to the long axis of the bone, between the two epiphyses (Figure 6.3A).

9. Tibial length: The condylomalleolar length was measured parallel to the long axis of the bone, between the superior aspect of the lateral tibial condyle and the most inferior tip of the medial malleolus (Figure 6.3B).
10. Heel height: The articulated height of the talus and calcaneus was measured from the most superior aspect of the trochlea, perpendicular to the plane of support, represented by a line drawn between the most inferior aspects of the head of the 5th metatarsal and the calcaneal tuberosity (Dayal et al., 2008), as illustrated in Figure 6.4.

Total skeletal height (TSH) was calculated by adding all the measurements, including cranial height, vertebral heights (C2-S1), lengths of the femur and tibia and the heel height (Fully, 1956). Based on the nature of immature remains found in forensic and/or archaeological contexts as well as the skeletal maturity thereof, different TSHs were calculated. This included the TSH-D calculated from the diaphyseal lengths of the femur and tibia as well as the TSH-M calculated from the maximum long bone lengths of both femur and tibia. Results indicated statistically significant differences between the maximum and physiological lengths of the femur ($p<0.0001$) and therefore the TSH-P was also calculated by combining the physiological length of the femur and the maximum length of the tibia.

6.2.2.3. Data analysis

The original examiner measured and re-measured 10 specimens in order to assess the reproducibility of the measurements collected from the MRI scans. In keeping with work by Bidmos and Manger (2012), one vertebra representing each of the vertebral regions was selected to assess the repeatability of the vertebral measurements and included C7, T10 and L5. The heights of the skull, C2, S1 and the heel were also re-measured along with the long

bone lengths of the femur and tibia. Repeatability of these measurements was tested using a Lin's concordance correlation coefficient (Lin, 1989).

Sub-adult males and females were assessed separately as significant sex differences with regards to the growth spurt and body proportions have been documented (Tanner, 1989; Nyati et al., 2006; Cole et al., 2015). The sexes were, however, also combined to increase the sample size and also to provide information that can be applied in forensic anthropological settings where sub-adult sex estimation remains controversial (Lewis & Rutty, 2003; Scheuer & Black, 2004).

All statistical analyses were carried out in SPSS, version 20. The minimum, maximum, mean and standard deviations were calculated to describe living stature as well as the computed variables (TSH-D, TSH-M, TSH-P). Differences between the sexes were tested with a student t-test and the correlations between the computed variables and living stature were assessed with a Pearson's product moment correlation. Following suggestions by Raxter et al. (2006) regression equations were computed to estimate living stature directly from the TSHs.



Figure 6.1. Illustration of the cranial and vertebral height measurements (C2-S1), scale = 5 cm.



Figure 6.2. Illustration of the A) diaphyseal, B) physiological and C) maximum length measurements of the femur, scale bare = 5 cm.

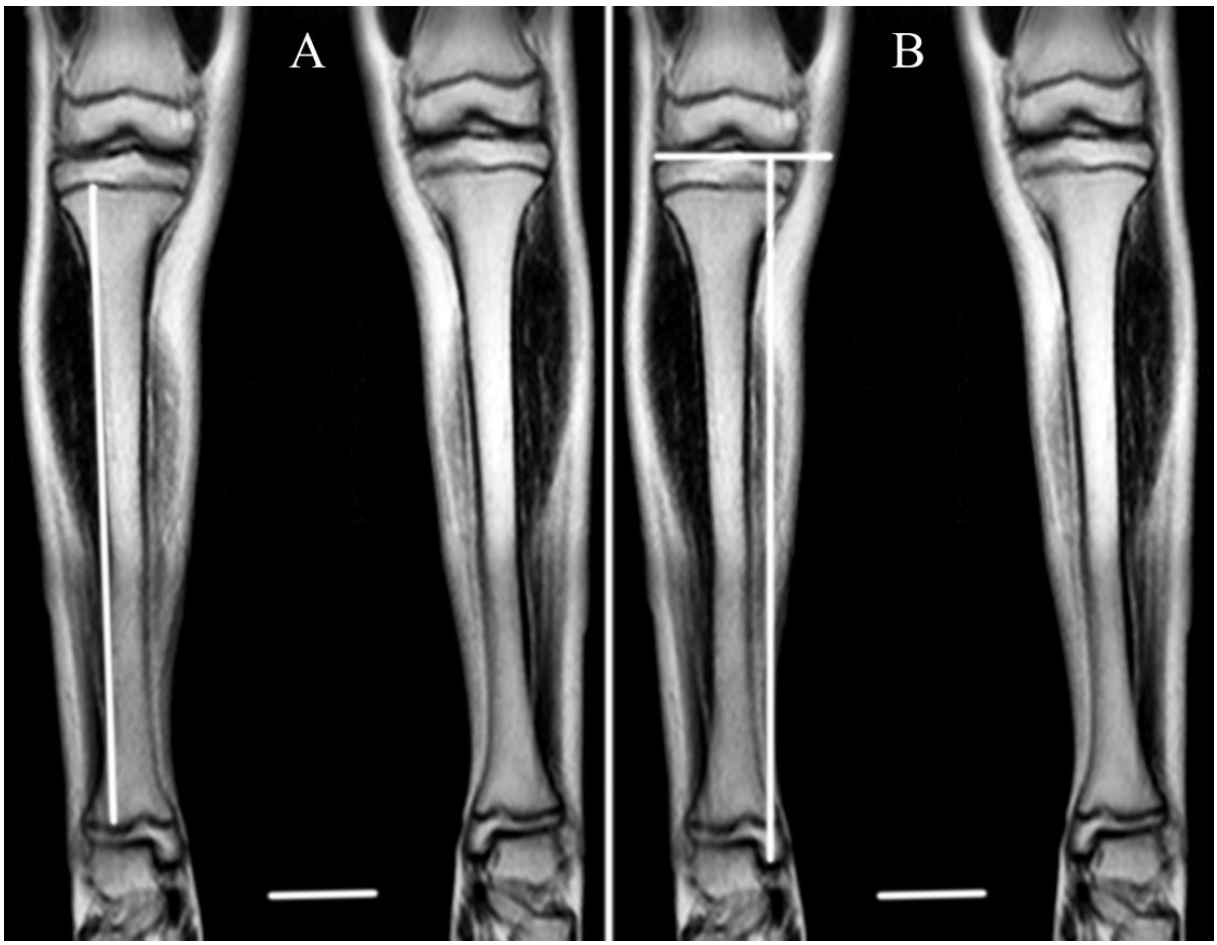


Figure 6.3. Illustration of the A) diaphyseal and B) maximum length measurements of the tibia, scale = 5 cm.



Figure 6.4. Image illustrating the talo-calcaneal measurements taken from the most superior aspect of the trochlea to a point on the plane of support, scale = 2 cm.

6.3. RESULTS

6.3.1. Repeatability

All the repeated measurements had Pc-values greater than 0.90 and were therefore deemed highly repeatable (Lin, 1989).

6.3.2. Descriptive statistics

Sub-adult males and females ranged between 10 and 17 year of age with an average age of 13.1 (± 2.0) years for sub-adult males, 13.3 (± 2.1) years for sub-adult females and 13.2 (± 2.1) years for the combined sex sample. Descriptive statistics including the minimum, maximum, mean and standard deviations for living stature and the TSHs are reported in Table 6.1. Living stature ranged between 129.0 and 173.3 cm (150.7 ± 12.7 cm) for sub-adult males and between 136.4 and 163.0 cm (150.7 ± 7.9 cm) for sub-adult females. The sub-adult male TSH measurements were characterized by large ranges and standard deviations, with sub-adult female TSH measurements being on average larger compared to that of sub-adult males. These differences were, however, not statistically significant ($p > 0.05$).

The nature and preservation of sub-adult skeletal remains retrieved for skeletal analysis depend in part on age-at-death which equates to the degree of fusion or non-fusion between the long bone diaphysis and the epiphyses. Therefore this study included measurements of the diaphysis to assist with skeletal analyses where the epiphyses are not yet fused as well as physiological and maximum long bone lengths where remains are found with the epiphyses attached or completely fused to the diaphysis.

6.3.3. Regression analyses

The correlations between living stature and the TSHs, along with the stature estimation regression equations for sub-adult males, females and a combined sex sample are summarized

in Table 6.2. All the correlations between living stature and the TSHs were very strong (r = 0.978 – 0.990) and statistically significant ($p < 0.0001$).

6.3.3.1. Total Skeletal Height – Diaphyseal Lengths (TSH-D)

The correlations between TSH-D and living stature was statistically significant ($p<0.0001$), with the strongest correlation observed for sub-adult males (r = 0.986). The correlation for sub-adult females and the combined sex sample was also very strong (r = 0.978). Scatter plots were included to visually assess the associations between living stature and the TSH-D for sub-adult males, sub-adult females and a combined sex sample (Figure 6.5). These scatter plots highlight the close distribution of the respective variables around the line of best fit.

The stature estimation regression equations calculated from the TSH-D were characterized by small standard error of estimates (SEEs). The SEE has been described as a measure of the accuracy of regression equations, with small SEEs indicating increased estimation accuracies (Bidmos, 2008a). The SEE for the equations derived from the TSH-D ranged from 1.76 cm to 2.19 cm. The SEE was smaller for the sub-adult female equations (mean SEE = 1.76 cm) compared to sub-adult male (mean SEE = 2.13 cm) and the combined sex sample (SEE = 2.19 cm) equations.

6.3.3.2. Total Skeletal Height – Physiological Lengths (TSH-P)

Scatter plots, illustrating the association between living stature and the TSH-P, are presented in Figure 6.6. These associations were characterized by very strong statistically significant ($p < 0.0001$) positive correlations, ranging between 0.981 and 0.990. The correlations between living stature and the TSH-P were slightly stronger than the correlations associated with the TSH-D. Again the strongest correlation was observed for sub-adult males

(r = 0.990) with similar correlations noted for sub-adult females and the combined sex sample (r = 0.981).

The stature estimation regression equations computed from the TSH-P were also characterized by small SEEs. These SEEs were smaller than the SEE calculated for the TSH-D regression equations. Sub-adult females had the smallest SEE (SEE = 1.58 cm), followed by sub-adult males (SEE = 1.82 cm) and the combined sex sample (SEE = 1.99 cm).

6.3.3.3. Total Skeletal Height – Maximum Lengths (TSH-M)

Results for the correlations between living stature and TSH-M are very similar to that reported for TSH-P. Again scatter plots were included to demonstrate these associations (Figure 6.7) and emphasize the strong relationship between the variables and the close distribution thereof around the line of best fit.

The SEE for the equations remained small; however, a small increase was noted for the TSH-M equations compared to the TSH-P equations, for both sub-adult males (SEE = 1.84 cm) and females (SEE = 1.59 cm). Interestingly, the SEE for the TSH-M equation (SEE = 1.93 cm) for the combined sex sample was smaller than reported for the TSH-P equation (SEE = 1.99 cm).

Overall the correlations between living stature and TSHs were stronger is sub-adult males compared to sub-adult females. Additionally, the SEEs for the stature estimation equations were smaller in sub-adult females compared to sub-adult males.

Table 6.1. Descriptive statistics, including the minimum (min), maximum (max), mean and standard deviation (SD), of the computed variables for sub-adult males, females and a combined sex sample.

	Males					Females					Combined				
Variables (cm)	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**	**n**	**Min**	**Max**	**Mean**	**SD**
Living stature	24	129.0	173.3	150.7	12.7	29	136.4	163.0	150.7	7.9	53	129.0	173.3	150.7	10.3
TSH-D[c]	24	103.6	141.5	122.0	10.9	27	110.4	137.0	123.6	7.8	51	103.6	141.5	122.8	9.3
TSH-P[d]	24	109.2	148.7	128.4	11.2	29	117.0	144.5	130.1	7.8	53	109.2	148.7	129.4	9.4
TSH-M[e]	24	109.5	149.2	128.8	11.3	29	117.2	144.6	130.3	7.8	53	109.5	149.2	129.6	9.5

[c]D – Diaphysis; [d]P – Physiological; [e]M – Maximum

Table 6.2. Pearson's product moment correlation coefficients (r) and regression equations for stature estimation in sub-adult males, females and a combined sex sample.

	Males					Females					Combined				
Variables (cm)	**r**	**r^2**	**Slope**	**Intercept**	**SEE**	**r**	**r^2**	**Slope**	**Intercept**	**SEE**	**r**	**r^2**	**Slope**	**Intercept**	**SEE**
TSH-D [c]	0.986	0.973	1.147	10.822	2.13	0.978	0.956	1.026	23.851	1.76	0.978	0.957	1.094	16.345	2.19
TSH-P [d]	0.990	0.980	1.120	6.849	1.82	0.981	0.962	1.002	20.194	1.58	0.981	0.963	1.068	12.553	1.99
TSH-M [e]	0.990	0.980	1.109	7.893	1.84	0.981	0.961	1.003	19.970	1.59	0.982	0.965	1.063	12.836	1.93

[c]D – Diaphysis; [d]P – Physiological; [e]M – Maximum

Significance at p<0.01 (2-tailed);

Estimated living stature = intercept + slope x variable ±SEE

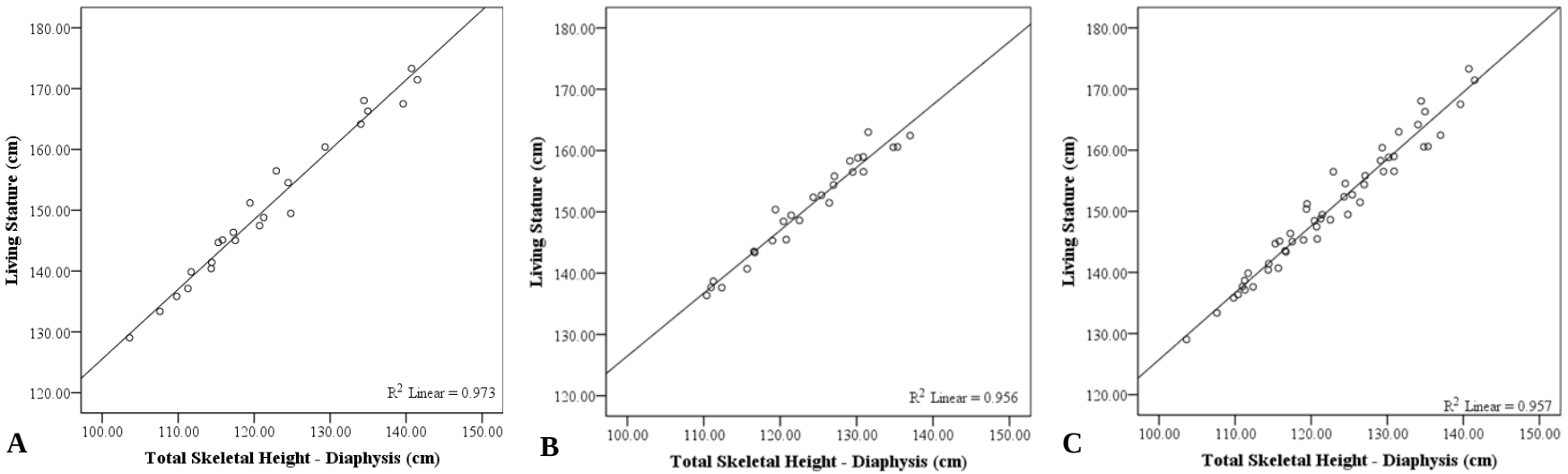


Figure 6.5. Scatter plots illustrating the association between living stature and the total skeletal height – diaphysis in A) sub-adult males, B) sub-adult males and C) a combined sex sample.

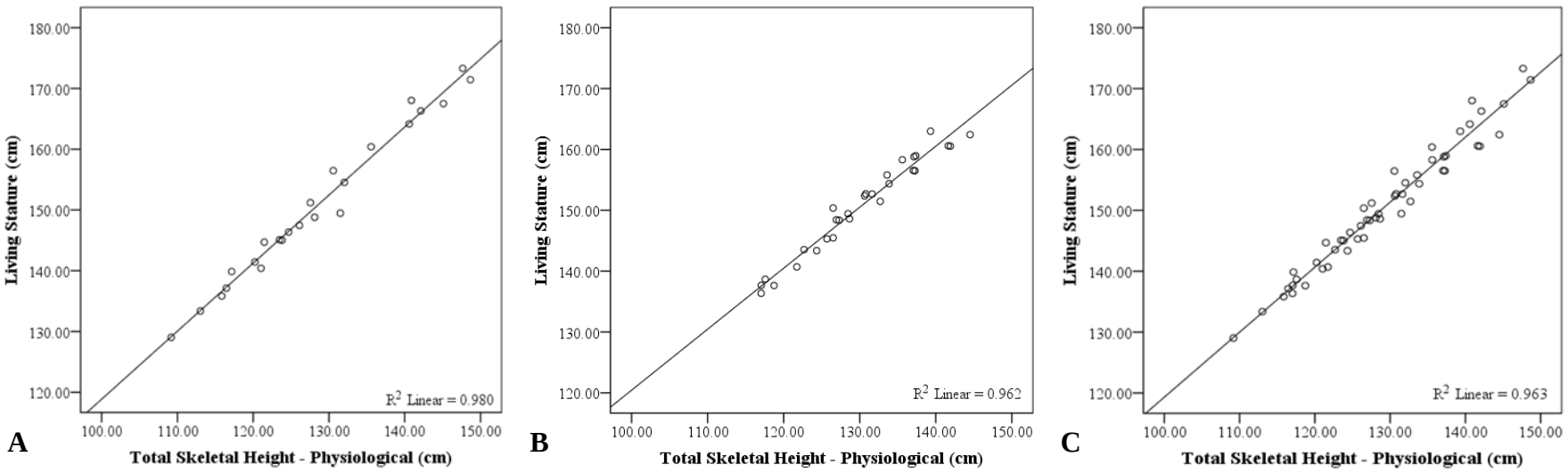


Figure 6.6. Scatter plots illustrating the association between living stature and the total skeletal height – physiological in A) sub-adult males, B) sub-adult males and C) a combined sex sample.

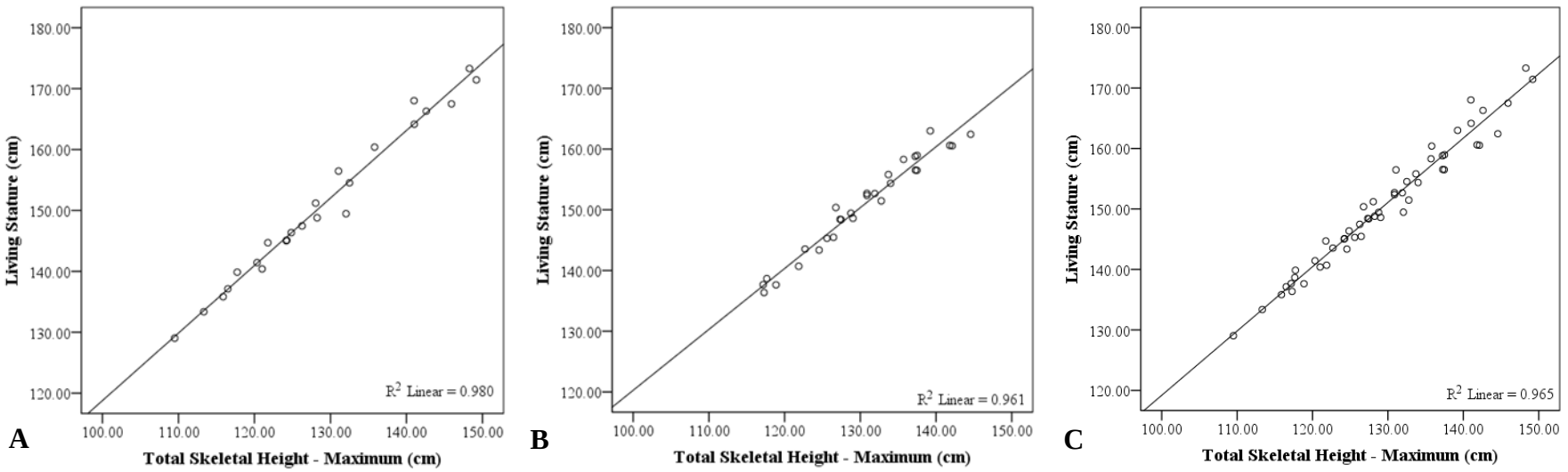


Figure 6.7. Scatter plots illustrating the association between living stature and the total skeletal height – maximum in A) sub-adult males, B) sub-adult males and C) a combined sex sample.

6.4. DISCUSSION

Descriptions of stature can provide vital information regarding growth, health, nutrition, socio-economic status and secular change (Kondo et al., 2000; Stulp & Barrett, 2014). It can also play a pivotal role in the positive identification of unknown skeletal remains (Ousley, 1995; Sjøvold, 2000; Smith, 2007; Moore & Ross, 2013). Methods regarding adult stature estimation are well documented; however, information regarding sub-adult stature estimation is few and far between. Sub-adult stature estimation from long bone lengths has been described but no attempts have been made to assess the anatomical method for sub-adult stature estimation. Therefore, the aim of this study was to describe the anatomical method for the estimation of stature in Black South African sub-adults.

Living stature of participants was recorded and allowed for the direct correlation of know heights with known bone length measurements. This afforded the opportunity to assess sub-adult stature estimation in a modern living population and as such excluded the unwanted effects of secular trends and the use of unreliable documented data. The Black South African sub-adult males from the current study were taller than Black South African sub-adult males measured in the Pretoria National Nutrition Survey (PNNS; 1963 – 1965) and the Birth-To-Twenty Project (Bt20; 1999 – 2001) and of similar heights compared to the White South African sub-adult males from these surveys. The Black South African sub-adult females from the current study were taller than both the Black and White South African sub-adult female participants assessed in the studies listed above (Hawley et al., 2009). These differences support secular change and the well documented increase in stature (Tanner, 1989; Hawley et al., 2009; Stulp & Barrett, 2014). The Black South African sub-adults were, however, shorter than their North-American (Fryar et al., 2012) and European counterparts, with the exception of a few age groups represented by very small sample sizes available for direct comparisons. These population differences can be explained by a number of genetic and environmental

factors including health, nutrition, seasonal and climatic variations, psychological stress, socio-economic status, secular trends and urbanization to list but a few (Eveleth & Tanner, 1976; Tanner, 1989; Stulp & Barrett, 2014).

The average TSHs were larger in sub-adult females compared to males. These differences indicate sex differences related to growth and skeletal maturation. Sub-adult females grow faster than sub-adult males by entering the adolescent growth spurt approximately two years prior to males and as such they also reach skeletal maturity earlier (Tanner, 1989; Cole et al., 2015). These growth differences between the sexes also produce differences related to body proportions (Eveleth & Tanner, 1976; Tanner, 1989). To our knowledge, no data on sub-adult TSHs are currently available for comparison; however, the sub-adult TSHs were smaller than the TSH reported for Black South African adult males also collected from MRI scans (Bidmos & Manger, 2012) and point towards the incomplete growth of the participants assessed in the current study.

Strong statistically significant positive correlations were observed between living stature and the TSHs. The correlations between TSHs and living stature were stronger than the correlations reported between living stature and sub-adult lower limb long bone lengths (Brits et al., 2016). The strong correlations related to TSHs are associated with the inclusion of all the skeletal elements that contribute towards living stature (Kondo et al., 2000; Raxter et al., 2006; Bidmos & Manger, 2012). The correlations between living stature and the TSHs for the Black South African sub-adult males, females and the combined sex sample were in fact greater than that reported for adult Black South African males ($r = 0.934$) (Bidmos & Manger, 2012) and North American populations ($r = 0.952$) (Raxter et al., 2006).

The SEEs for the stature estimation models from the TSHs was smaller than that recorded for adult North Americans (SEE = 2.31 cm) (Raxter et al., 2006). The SEEs for sub-adult females were also smaller than that reported by Bidmos & Manger (2012) for Black South

African adult males (SEE = 1.93 cm) and comparable to that calculated for sub-adult males. These smaller SEEs for the TSH measurements are related to the inclusion of all the supero-inferior skeletal elements that contribute directly to stature and as such eliminate the errors introduced by differences in body proportions (Lundy, 1985; Kondo et al., 2000; Raxter et al., 2006). The smaller SEEs noted in sub-adult females again point to the differences observed in the timing and duration of the growth spurt (Eveleth & Tanner, 1976; Tanner, 1989; Cole et al., 2015). From the standard deviations (SD) it is also clear that there is less variance in the sub-adult female sample compared to the sub-adult males and as such the stature estimation regression equations are more accurate in sub-adult females compared to males.

6.5. CONCLUSION

This study aimed to describe the estimation of stature in sub-adults using the anatomical method by employing modern image modalities to overcome the shortage of sub-adult skeletal collection.

The results indicated very strong, statistically significant positive correlations between living stature and TSHs. The sub-adult stature estimation accuracies of the anatomical method outweighed that of regression equations derived from long bone lengths and produced accuracies comparable to that reported for adults. The use of the sex specific regression equations is promoted; however, the regression equations derived from the combined sex sample can also be used, with similar accuracies, when sex is unknown.

The anatomical method is therefore encouraged for stature estimation in sub-adults as it will add valuable information when dealing with unknown sub-adult skeletal remains. The use of this method is, however, limited to the presence and preservation of the skeletal elements that contribute directly to stature. The small sample size, representing only adolescents restricts the application of this method and therefore future research should put effort toward describing

the anatomical method in larger samples, stratified according to sex, population and age as differences in growth between ages, sexes and populations are evident.

CHAPTER SEVEN

Discussion and Conclusion

7.1. INTRODUCTION

Stature estimation from skeletal remains plays an important role in bioarchaeology and paleoanthropology and can provide valuable information pertaining to growth and development, health, nutrition, socio-economic status, sexual dimorphism and secular change to name but a few. Estimates of stature also play a valuable role in forensic anthropology and offer descriptions of the deceased which can be used for positive identification, or exclusion of an individual, and as such forms an important part of the biological profile (Jungens, 1988; Feldesman et al., 1990; Ousley, 1995; Kondo et al., 2000; Sjøvold, 2000; Moore & Ross, 2013; Stulp & Barrett, 2014; Cardoso et al., 2016). Stature has been described as the "most straightforward parameter to estimate from human skeletal remains" (Cardoso et al., 2016, p. 55); however, stature is affected by a number of factors including age, ancestry, sex, secular change and diurnal variation (İşcan & Steyn, 2013; Moore & Ross, 2013; Cardoso et al., 2016). Stature estimation methods are therefore sex-, population- and century-specific and are continuously reviewed (Baines et al., 2011; Cardoso et al., 2016).

Reviews of the anatomical methods have illustrated that this method significantly underestimates stature and therefore the first aim of this study was to assess the accuracy of the anatomical method for the estimation of stature in living Black South African females using MRI scans.

The second aim of the studies undertaken was to describe stature estimation in sub-adults. Stature is rarely estimated when dealing with sub-adult skeletal remains due to the general lack of standards and methods available (Feldesman, 1992; Kondo et al., 2000; Lewis & Rutty, 2003; Smith, 2007; Cardoso, 2009; Sutphin & Ross, 2011; İşcan & Steyn; 2013). This is due to the lack of sub-adult skeletal collections available for research (Telkkä et al., 1962; Lewis & Rutty, 2003; İşcan & Steyn; 2013), which was circumvented in the current study by using MRI scans of living participants.

7.2. MAGNETIC RESONANCE IMAGING (MRI) SCANS

Uncertainty regarding the accuracy and reliability of skeletal measurements collected from imaging modalities remains a cause of concern. Therefore the aim of chapter three was to assess the accuracy and repeatability of skeletal measurements collected from MRI scans.

Results indicated high intra- and inter-observer repeatability, indicating that skeletal measurements can easily be collected from MRI scans. This was further supported by the high intra- and inter-observer repeatability also noted for the skeletal measurements collected in chapters four, five and six.

Statistically significant differences were found between dry and wet bone measurements and were linked to the presence of cartilage and adhering soft tissue. No significant differences were found between dry bone and MRI measurements, except for the epicondylar breadth of the femur. This difference is thought to be related to difficulties associated with MRI scans collected from cadaveric remains with insufficient surrounding soft tissue. The mean differences between dry bone and MRI measurements recorded in the current study were less than 2 mm, which is within the range of error accepted by forensic anthropologists (e.g. Stull et al., 2013, 2014b). These results demonstrated the accuracy of skeletal measurements collected from MRI scans and as such MRI can be considered as an alternative means for collection of osteometric data.

7.3. ADULT STATURE ESTIMATION

Studies have found that the anatomical method significantly underestimates stature (King, 2004; Bidmos, 2005; Raxter et al., 2006; Maijanen, 2009; Bidmos & Manger, 2012). This underestimation is thought to be caused by the use of universal soft tissue correction factors provided by Fully (1956) (King, 2004; Bidmos, 2005; Raxter et al., 2006), which some

authors propose could be sex- and population-specific (King, 2004; Bidmos, 2005; Bidmos & Manger, 2012).

Results from chapter four indicated that the anatomical method, as described by Fully (1956) and Raxter et al. (2006), significantly underestimate stature, while the method described by Bidmos & Manger (2012) in Black South African males significantly overestimate stature in Black South African females. These observations raised questions regarding the applicability of the soft tissue correction factor proposed by these authors. The soft tissue correction factor calculated for Black South African females were larger than the soft tissue correction factor described for Europeans (Fully, 1956) and North Americans (Raxter et al., 2006) and supports the population specificity of these correction factors as suggested by King (2004) and Bidmos (2005). The soft tissue correction factor calculated for Black South African females was significantly smaller than calculated for Black South African males, and as such also confirms the sex specificity of these soft tissue correction factors. The results also refuted the correlation between the soft tissue correction value and total skeletal height as described by Fully (1965).

Based on these inaccuracies, regression analysis was used to create a new stature estimation model for Black South African females from the TSH derived from MRI scans. This model was characterized by a very strong statistically significant positive correlation with living stature ($r = 0.942$) and a small SEE (1.80 cm). Moreover, 95% of stature estimates calculated using the new model fell within two standard errors of the estimate and further attests to the accuracy of the newly proposed model.

Raxter et al. (2006) proposed that age had an effect on the accuracy of stature estimation, but in keeping with results by Trotter and Gleser (1952) and Cline et al. (1989), this effect was not significant for individuals in the current study which consisted of individuals younger than 60 years of age.

7.4. SUB-ADULT STATURE ESTIMATION

There is a significant paucity of research on sub-adult stature estimation. This is related to the absence of large, modern, sub-adult skeletal collections with known demographic information (Telkkä et al., 1962; Lewis & Rutty, 2003; İşcan & Steyn, 2013). To overcome this difficulty, the current study examined stature estimation in sub-adults based on skeletal measurements taken from MRI scans of living Black South African sub-adults.

7.4.1. Mathematical method

The mathematical method is the most commonly used stature estimation method as it makes provision for the estimation of stature from a single bone or from combination of bones (Tatarek et al., 2005). Therefore chapter five described the correlation between stature and lower limb long bone lengths and also derived regression equations for the estimation of stature in Black South African sub-adults.

The maximum and diaphyseal lengths of the femur and tibia as well as the combined measurements were compared to living stature in 59 Black South African sub-adults. Results indicated very strong statistically significant positive correlations for sub-adult males, females and a combined sex sample. The correlations along with the SEEs of the regression equations were comparable to correlations and SEEs reported by Telkkä et al. (1962), Himes et al. (1977), Ruff (2007), Smith (2007), Abrahamyan et al. (2008), Banik et al. (2012) and Song-in (2013) for sub-adult long bone measurements, but exceeded the correlations found between stature and measurements of the foot (Grivas et al., 2008; Krishan et al., 2011, 2012b), fingers (Krishan et al., 2012a), hand (Ibegbu et al., 2015) and head (Vinitha et al., 2015). Furthermore, the correlations between the long bone measurements and stature were comparable to that reported for Black South African adults, while the SEEs for the sub-adult stature estimation models were slightly larger than that of Black South African adults (Lundy & Feldesman, 1987; Dayal

et al., 2008). These measurements can therefore be considered good estimators of sub-adult stature.

7.4.2. Anatomical method

Estimating stature from a complete skeleton produces more accurate estimates, as variation in body proportions are inherently incorporated in the skeletal measurements (Lundy, 1985). To date the anatomical method has not been considered for stature estimation in sub-adults and therefore chapter six assessed the use of this method for stature estimation in Black South African sub-adults.

All the skeletal elements that contribute directly to stature were measured from 53 full body MRI scans representing Black South African sub-adults. Standardized measurements from these elements were obtained and summed to calculate the TSH. Following suggestions by Raxter et al. (2006) the TSH was used in combination with regression analyses to produce sub-adult stature estimation equations. The results indicated very strong statistically significant positive correlations ($r = 0.978$ to $r = 0.990$) between living stature and the TSH for sub-adult males, females and a combined sex sample. The obtained regression equations were characterized by small SEEs. These correlations along with the SEEs outweighed that reported for the lower limb long bone measurements in chapter five. These stronger correlations and smaller SEEs are related to the inclusion of all the skeletal elements contributing to living stature (Lundy, 1985; Kondo et al., 2000; Raxter et al., 2006). Moreover, the correlations reported in the current study were stronger, while the SEEs were smaller or comparable to, that reported for adult stature estimation equations from the TSH (Raxter et al., 2006; Bidmos & Manger, 2012).

Estimating sub-adult stature using the anatomical method is therefore strongly recommended when a complete or nearly complete skeleton is retrieved.

7.5. LIMITATIONS AND FUTURE DIRECTIONS

MRI affords the opportunity to study skeletal remains of living individuals thereby avoiding problems such as collection bias (Komar & Grivas, 2008) and missing or incorrect cadaver demographics (Lundy, 1983; Bidmos, 2005). Studying living individuals also ensures that a current population is examined and circumvents problems such as secular changes often associated with skeletal collections (Meadows & Jantz, 1995; Ousley & Jantz, 1997; Meadows Jantz & Jantz, 1999; Dirkmaat et al., 2008; Komar & Grivas, 2008). This study further benefitted from collection of living stature data from participants as opposed to the use of cadaveric height, which has been found to overestimate living stature (Cardoso, 2009).

The conclusions drawn from this study are, however, limited by a number of factors such as the small sample sizes available for analysis. This is mainly due to the increased costs related to MRI scanograms as well as the prolonged scan time (Leitzes et al., 2005; Rathnayaka et al., 2012). Future studies would greatly benefit from increased sample sizes for statistical analyses.

Results from chapter three illustrated no statistically significant differences between MRI and lower limb dry bone measurements. Unfortunately, only cadaver lower limbs were available for analysis and future studies would greatly benefit from assessing the differences between MRI and dry bone measurements of other skeletal elements such as the skull and vertebrae, as difficulties related to the accurate measurement of these elements from imaging modalities have been noted (Ruff et al., 2012). Furthermore, with the increased popularity of skeletal analysis from virtual images, future studies should put efforts toward establishing measurement protocols that adhere to the Daubert criteria (Dirkmaat et al., 2008).

In line with reports by King (2004), Bidmos (2005), Raxter et al. (2006) and Bidmos & Manger (2012) results from the current study showed that Fully's (1956) method significantly underestimates stature in Black South African females. Fully's (1956) method is routinely used

to generate regression equations for the estimation of stature from various bones and bone combinations in South Africa due to missing or questionable documented cadaveric heights (Lundy, 1983, 1988; Bidmos, 2005; Dayal et al., 2008). The inaccuracies demonstrated here therefore calls into question the accuracies of these regression equations and future efforts should concentrate on assessing the accuracies of these methods and produce new equations if needed.

The sub-adult stature estimation equations calculated in the current study are limited to Black South African sub-adults aged between 10 and 17 years, with living statures ranging between 129.0 cm and 173.3 cm. Future studies will greatly benefit from increased sample sizes, representing sub-adults of various heights, ages and population groups, separated by sex. Additionally, the stature estimation equations produced in the current study are limited to the lower limb long bones and efforts towards the upper limb bones and other skeletal elements are encouraged. Research on sub-adult stature estimation will also greatly benefit from increased knowledge based on bone epiphyses and other soft tissue element that can affect the accuracy of stature estimates.

The stature estimation methods described here were derived from skeletal measurements collected from MRI scans and before these methods are accepted as standard practice, it should be tested on skeletonized remains.

7.6. FINAL CONCLUSIONS

Results from this study support the following main conclusions:

1. There are no statistically significant differences between the osteometric measurements collected from MRI scans and that collected from dry bones, except for the epicondylar breadth of the femur.
2. Measurements collected from MRI scans are easily reproducible.

3. The universal soft tissue correction factor, which is used in the calculation of living stature from TSH, is not applicable to Black South Africans as this value underestimates living stature.
4. Results indicate that the soft tissue correction factor is sex- and population specific.
5. There are very strong statistically significant positive correlations between living stature and the maximum and diaphyseal lengths of the femur and tibia as well as the TSH of sub-adults.
6. Sub-adult stature estimation regression equations are characterized by small SEEs, comparable to that of adults.
7. The anatomical method produces more accurate sub-adult stature estimates compared to regression equations derived from lower limb long bones.

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APPENDIX A

Human Research Ethics Committee (Medical) –

Clearance Certificate No. W-CJ-140604-1

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265

Ref: W-CJ-140604-1 04/06/2014

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof T J M Daly).

Project title: research on cadaveric material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health research. Use of such material is subject only to permission from the responsible person in the School of anatomical Sciences – the Head or person designated by the Head.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2014 -06- 04
JOHANNESBURG

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

Copy - HREC(Medical) Secretariat : Anisa Keshav, Zanele Ndlovu.

APPENDIX B

Research Contribution

2016/02/29

Research Contribution

The research described in Chapter 1 entitled "The accuracy and repeatability of skeletal measurements collected from Magnetic Resonance Imaging Scans" was completed in association with Ms. Shayla Pillay and Dr. Temitope Essan.

I, Desiré M Brits, student number, 555287 declare that I contributed adequately towards the research and research findings being prepared for publication which are included in my thesis. I was responsible for the conception of the research as well as the funding. I was also involved in the data collection, data analyses, data interpretation and write-up of this research.

Ms. Pillay was involved in data collection, data analysis, data interpretation and write up while Dr. Essan was involved in the data analysis and data interpretation. Dr. Essan also gave valuable input in the write-up of this research.

The co-authors hereby agree to the use of this research by the student as part of her thesis.





D. Brits S. Pillay T. Essan

APPENDIX C

Human Research Ethics Committee (Medical) –

Clearance Certificate No. M110414

R14/49 Ms Desire M Brits

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M110414

NAME: (Principal Investigator) Ms Desire M Brits

DEPARTMENT: School of Anatomical Sciences
Medical School

PROJECT TITLE: Stature Estimation in Indigenous South African during Growth

DATE CONSIDERED: 06/05/2011

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof P Manger

APPROVED BY: _____
Professor P E Cleaton-Jones, Chairman HREC (Medical)

DATE OF APPROVAL: 06/07/2011

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D

Adult Participant Information Leaflet and Informed Consent

INFORMATION LEAFLET AND INFORMED CONSENT – ADULT VOLUNTEERS

Study title:

Stature estimation in South African African juveniles and adult females

Good day, my name is Desiré Brits. I am a researcher in the School of Anatomical Sciences at the University of the Witwatersrand, Johannesburg. I am doing research on the identification of human remains that have been skeletonised. I would like to use Magnetic Resonance Imaging (MRI) scans of healthy individuals to help me develop stature estimation tools.

What are we asking you to do?

We are asking you to volunteer to have a full body MRI taken at the Wits-Donald Gordon Medical centre. You would be expected to change into a theatre gown after which your height and weight would be measured. We would then ask you to lie as still as possible on the MRI scanning bed for approximately 20 min while the scan is taken. The whole process should not take up more than an hour of your time. The MRI's will be loaded and reconstructed on a computer after which we will take measurements on the bones for statistical analysis.

Please read the following statements before making your decision to help us.

1. If you agree to take part in this study, you would be required to complete the attached form. We need details of your medical and surgical history to see if you qualify for this study.
2. If you agree to take part in this study, you may be examined for any abnormalities of the back (spine) and other parts of the skeletal system to determine if you qualify to participate in this study.
3. Your gender, date of birth and handedness will also be asked for and you may need to provide an Identification Document (ID) or birth certificate to confirm your age.
4. If you agree to take part in this study, you would be transported to the Wits-Donald Gordon Medical Centre by me.
5. If you have any questions, please do not hesitate to ask me, Desiré Brits or any of my supervisors.
6. You should not agree to take part unless you are satisfied and comfortable with all the procedures involved.
7. Please be open with us regarding your health history as it could be harmful to you if you don't.
8. If you decide to take part in this study, you would be asked to sign this document to confirm that you understand the study. You will also be given a copy to keep.

Will this study procedures result in discomfort or inconvenience?

Magnetic Resonance Imaging (MRI) is a safe diagnostic procedure with no known side-effects. The procedure does not use any form of radiation and is therefore safe. MRI uses a strong static and pulsed gradient magnetic field and radiofrequency energy to produce images. These magnetic fields could cause metallic objects such as pacemakers or cochlear implants to move or heat up and therefore you can not undergo a MRI if you have any metal objects or implants in your body. MRI scanning is also noisy and some people have complained of claustrophobia (which is the fear of enclosed spaces). For your piece of mind, qualified and experienced personnel will perform the scans.

Benefits:

- You may not benefit directly from this study.
- However, your participation in this study will contribute to the development of the field forensic science in South Africa which may assist law enforcement agencies in identifying individuals from their skeletal remains.

You may not take part in this study if:

- You are pregnant or breastfeeding or planning to become pregnant during the study period. Please sign the *Supplementary Informed Consent for Women of Childbearing Potential (12 Years and Older),* at the end of this document.
- You have suffered from nutritional diseases such as kwashiorkor.
- You have suffered from any bone affecting diseases.
- You have broken any bone in your body within the past year.
- You have answered yes to any of the questions on the MRI exclusion criteria list compiled by Wits-Donald Gordon Medical Centre.

Rights as a participant in this study:

- Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without giving a reason. Your withdrawal will not affect you in any way.
- We also retain the right to withdraw you from this study at any time if it is considered to be in your best interest or if we realise that you were not honest on the medical history form.

Financial arrangements:

- There will be no financial cost to you as a participant of this study unless you prefer to drive to the Wits-Donald Gordon Medical Centre yourself.
- You will not be paid for your participation in this study.

Ethical approval:

- This study's protocol has been approved by the University of the Witwatersrand, **Human Research Ethics Committee (HREC, Medical)**.
- If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

Confidentiality:

- All information obtained during the course of this study, including personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals or at scientific conferences will not include any information that could identify you as a participant in this study.
- The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC), the South African Medicines Control Council (MCC) and/or the United States Food and Medicine Administration (FDA). Therefore, you hereby authorise us to release your personal information to the employees or agents, of these authorities.

- The MRI's would be stored at the School of Anatomical Sciences and may be used for future research subject to approval of the **Human Research Ethics Committee (HREC, Medical)**.

Please contact me if you have any questions or want additional information about the procedures.
Desiré Brits: 011 717 2304 (office hours) or 084 585 2215 (any time)
Alternatively you can contact the School secretary Ms. Lizzie Marole at 011 717 2305 during office hours.

CONSENT FORM

I hereby confirm that I have been informed by the investigators, Desiré Brits, about the nature, conduct, benefits and risks of this study:

Stature estimation in South African African juveniles and adult females.

I understand that I may not participate in this study if I have any metal implants or objects in my body or if I am claustrophobic (fear of small spaces).
I have also received, read and understood the above written information regarding this study.
I am aware that the results of this study, including personal details regarding my sex, age, population affinity, height, date of birth and handedness will be anonymously processed into a study report.
I may, at any stage, without prejudice, withdraw my consent and participation in this study.
I have had sufficient opportunity to ask questions and (of my own free will) declare myself willing to participate in the study.

PARTICIPANT:

Printed Name and Surname Date and Time

Signature / Mark or Thumbprint

SUPPLEMENTARY INFORMED CONSENT FOR WOMEN OF CHILDBEARING POTENTIAL (±12 YEARS AND OLDER):

- I understand that as I am a woman of childbearing potential and in order to participate in this study, I should not be pregnant or breastfeeding.
- I must have a negative pregnancy test before I can enter this study.
- I understand that MRI scans is diagnostic and its side-effects on the fetus are unknown.
- This supplementary informed consent has been explained to me to my satisfaction.
- I have been given the chance to ask questions and they have been answered to my satisfaction.
- I hereby voluntarily consent to participate in this study.
- I understand that I will receive a copy of this supplementary consent form.

PARTICIPANT:

Printed Name and Surname Date and Time

Signature / Mark or Thumbprint

APPENDIX E

Information Leaflet and Informed Consent for

Parents of Child Volunteers

INFORMATION LEAFLET AND INFORMED CONSENT – CHILD VOLUNTEERS

Study title:

Stature estimation in South African African juveniles and adult females

Good day, my name is Desiré Brits. I am a researcher in the School of Anatomical Sciences at the University of the Witwatersrand, Johannesburg. I am doing research on the identification of human remains that have been skeletonised. I would like to use Magnetic Resonance Imaging (MRI) scans of healthy individuals to help me develop stature estimation tools.

What are we asking your child to do?

We are asking your child to volunteer to have a full body MRI taken at the Wits-Donald Gordon Medical Centre. Your child would be expected to change into a theatre gown after which his/her height and weight would be measured. We would then ask him/her to lie as still as possible on the MRI scanning bed for approximately 20 min while the scan is taken. The whole process should not take up more than an hour of your child's time. The MRI's will be loaded and reconstructed on a computer after which we will take measurements on the bones for statistical analysis.

Please read the following statements before making your decision to allow your child to volunteer for this study.

1. If you and your child agree to take part in this study, you would be required to complete the attached form. We need details of your child's medical and surgical history to see if he/she qualifies for this study.
2. If you and your child agree to take part in this study, your child may be examined for any abnormality of the back (spine) and other parts of the skeletal system to determine if he/she qualifies to participate in this study.
3. Your child's gender, date of birth and handedness will also be asked for and you may need to provide an Identification Document (ID) or birth certificate to confirm your child's age.
4. If you and your child agree to take part in this study, you would be transported to the Wits-Donald Gordon Medical Centre.
5. If you or your child has any questions, please do not hesitate to ask me, Desiré Brits or any of my supervisors.
6. You and your child should not agree to take part unless you and your child are satisfied and comfortable with all the procedures involved.
7. Please be open with us regarding your child's health history as it could be harmful to him/her if you don't.
8. If you and your child decide to take part in this study, you would be asked to sign this document to confirm that you understand the study. You will also be given a copy to keep.

Will this study procedures result in discomfort or inconvenience?

Magnetic Resonance Imaging (MRI) is a safe diagnostic procedure with no known side-effects. The procedure does not use any form of radiation and is therefore safe. MRI uses a strong static and pulsed gradient magnetic field and radiofrequency energy to produce images. These magnetic fields could cause metallic objects such as pacemakers or cochlear implants to move or heat up and therefore your child cannot undergo a MRI if they have any metal objects or implants in their body. MRI scanning is also noisy and some people have complained of claustrophobia (which is the fear of enclosed spaces). For your piece of mind, qualified and experienced personnel will perform the scans.

Benefits:

- Neither you nor your child may benefit directly from this study.
- However, your child's participation in this study will contribute to the development of the field of forensic science in South Africa which may assist law enforcement agencies in identifying individuals from their skeletal remains.

Your child may not take part in this study if:

- He/she have suffered from nutritional diseases, such as kwashiorkor.
- He/she have suffered from any bone affecting diseases.
- He/she have broken any bone in their bodies within the past year.
- You have answered yes to any of the questions on the MRI exclusion criteria list compiled by Wits-Donald Gordon Medical Centre.

Rights as a participant in this study:

- Your child's participation in this study is entirely voluntary and you and or your child can decline to participate, or stop at any time, without giving a reason. Your and/or your child's withdrawal will not affect either of you in any way.
- We also retain the right to withdraw your child from this study at any time if it is considered to be in his/her best interest or if we realise that you were not honest on the medical history form.

Financial arrangements:

- There will be no financial cost to you if your child participates in this study unless you prefer to drive your child to the Wits-Donald Gordon Medical Centre yourself.
- Neither you nor your child will be paid for participating in this study.
- Food and refreshments will be provided for children that undergo scans on Saturdays and during School holidays and a R100 reimbursement for any incidental costs incurred will also be given.

Ethical approval:

- This study's protocol has been approved by the University of the Witwatersrand, **Human Research Ethics Committee (HREC, Medical)**.
- If you want any information regarding your child's **rights as a research participant, or complaints regarding these research studies**, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

Confidentiality:

- All information obtained during the course of this study, including personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals or at scientific conferences will not include any information that could identify your child as a participant in this study.
- The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC), the South African Medicines Control Council (MCC) and/or the United States Food and Medicine Administration (FDA). Therefore, you hereby authorise us to release your child's personal information to the employees or agents, of these authorities.

- The MRI's would be stored at the School of Anatomical Sciences and may be used for future research subject to approval of the **Human Research Ethics Committee (HREC, Medical)**.

Please contact me if you have any questions or want additional information about the procedures.
Desiré Brits: 011 717 2304 (office hours) or 084 585 2215 (any time)
Alternatively you can contact the School secretary Ms. Lizzie Marole at 011 717 2305 during office hours.

CONSENT FORM

I hereby confirm that I have been informed by the investigator, Desiré Brits and/or Mandisa Tshambula, about the nature, conduct, benefits and risks of the study:

Stature estimation in South African African juveniles and adult females.

I understand that my child may not participate in this study if they have any metal implants or objects in their body or if they are claustrophobic (fear of small spaces).
I have also received, read and understood the above written information regarding this study.
I am aware that the results of this study, including personal details regarding the sex, age, population affinity, height, date of birth and handedness of my child will be anonymously processed into a study report.
I may, at any stage, without prejudice, withdraw my consent and child's participation in this study.
I have had sufficient opportunity to ask questions and (of my own free will) declare myself willing to allow my child to participate in this study with his/her consent.

PARENT/LEGAL GUARDIAN:

Printed Name and Surname Date and Time

Signature / Mark or Thumbprint

APPENDIX F

Assent Form for Child Participants

INFORMATION LEAFLET AND ASSENT FORM – PARTICIPANT ± 7 YEARS AND OLDER

Good day, my name is Desiré Brits. I am a researcher at the University of the Witwatersrand and I am doing research on human bones. I would like to use a medical procedure called Magnetic Resonance Imaging (MRI) to study the bones of healthy children so that I can create techniques to determine how tall they are.

What would we like you to do?
We would like to ask you to volunteer for this study. If you agree to take part, we would like to take you to Wits-Donald Gordon Medical Centre where a full body MRI scan of your body will be taken. At the Medical Centre we would ask you to change into a theatre gown for the scan and also so we can determine how tall you are and how much you weigh. We would ask you for some personal information such as your date of birth. We would then ask you to lie as still as possible on the MRI scanning bed which consists of a small tunnel, for approximately 20 minutes while a picture of your bones are taken. The MRI machine uses magnetic fields to produce images that could cause metallic objects to move or heat up. It may also be a little noisy, but the people at the Medical Centre will put "earphones" on year ears to make it less noisy. The MRI scans will be saved on a CD and measurements of the bones will be taken on a computer. If you agree to take part in this study, your parents will also need to give permission for your participation. You do not have to take part in this study and you can even stop at anytime if you do not want to continue being part of this study.

Unfortunately there are a few things that might prevent you from taking part in this study. These things include the following:
- If you know or suspect that you may be pregnant.
- If you have suffered from nutritional diseases such as kwashiorkor.
- If you have fractured or broken any bone in your body within the past year.

You can ask me any questions about the study to make sure that you understand everything I would like you to do.

Please contact me if you have any questions
Desiré Brits: 011 717 2304 (office hours) or 084 585 2215 (any time)

Thanks!

ASSENT FORM

I hereby agree to take part in the study called Stature estimation in South African African juveniles and adult females

I confirm that I have been informed by Desiré Brits and/or Mandisa Tshambula, about this study and I am comfortable with the intended procedures.
I understand that I may not participate in this study if I have any metal implants or objects in my body or if I am claustrophobic (fear of small spaces).
I acknowledge that my participation is voluntary and that I may withdraw from this study at any time when I feel uncomfortable or without reason.

PARTICIPANT:

Printed Name and Surname	Signature	Date

YOUNG LADIES OF CHILD BEARING AGE (12 to 17 years)

- I understand that as I am old enough to have a baby, and to participate in this study I should not be pregnant or breastfeeding.
- I also understand that if I participate in this study while pregnant I could potentially harm the baby.
- I hereby confirm that I am not pregnant and voluntarily agree to participate in this study.

If you are unsure if you are pregnant, please speak to me.

PARTICIPANT:

Printed Name and Surname	Signature	Date

APPENDIX G

Turn-it-in Digital Receipt

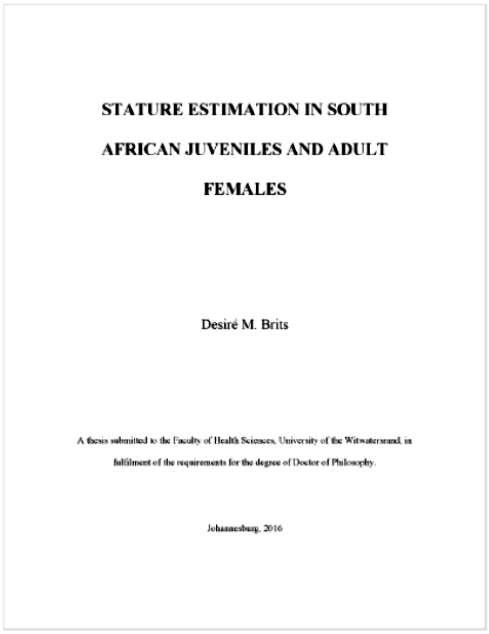
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STATURE ESTIMATION IN SOUTH AFRICAN JUVENILES AND ADULT FEMALES

Desiré M. Brits

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2016

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