CANCELLOUS BONE USE IN THE ESTIMATION OF SEX IN A SOUTH AFRICAN CADAVERIC AND FORENSIC SAMPLE by

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Abstract

The increasing number of unidentified individuals has become a growing concern worldwide. These individuals, whose identities remain unknown following medico-legal autopsy, present a complex challenge for authorities and communities alike. Forensic anthropology is a valuable tool to assist in the identification process, however further research is necessary to alleviate the increasing number of unidentified individuals. Imaging techniques such as X-rays and CT scanning have become an avenue in which research into the field of anthropology has expanded. LODOX imaging finds itself in an ideal position due to its widespread availability in most mortuaries in South Africa, primarily used for trauma analysis and identification of foreign objects. The overall aim of the study is to assess radiographic and dry bone measurements of the proximal femur for sex estimation in a South African population. This was done by assessing radiographic and dry bone parameters (e.g., traditional morphometric parameters and assessment of cancellous area and indirect density) for association with sex. Femora (58 males and 32 females) from the University of Cape Town Skeletal Repository were included in the study. Models generated were applied to a forensic cohort. Seventeen of the twenty-four variables included in this study were found to be significantly associated with sex. To determine the best possible model for sex estimation, an all-sub-sets logistic regression was performed. The two best models (model 1: cancellous area; model 2: cancellous area, maximum length and physiological length) that were generated, both showed an 88.2% agreement with previous sex estimates when applying them to a new cohort.

Table of Contents

List of figures

List of tables

List of Abbreviations

Abbreviation	Full Word
AIC	Akaike's information criterion
AP	Anteroposterior
BIC	Bayesian information criterion
BMD	Bone mineral density
BV/TV	bone volume/total volume
CT	Computed tomography
CI	Confidence interval
DEXA	Dual-energy X-ray absorptiometry
FA	Fractal analysis
FACT	Forensic Anthropology Cape Town
FPS	Forensic Pathology Services
GT-FC	The length greater trochanter to the fovea capitis
HREC	Human Research Ethics Committee
ICC	Intraclass correlation coefficient
IL	Illinois
LDA	Linear Discriminant Analysis
LLDA	Laser Linear Densitometric Analyses
LODOX	Low dose x-rays
MCS	Maximum Compressive Strength
MDCT	Multi-detector computed tomography
MSS	Maximum sheer stress
ML	Mediolateral
PMCT	Post-mortem computer tomography
PMI	Postmortem interval
QCT	Quantitative computed tomography
ROI	Region of Interest
TEM	Technical error of measurement
UCT	University of Cape Town
USA	United States of America
vBMD	Volumetric bone mineral density

Chapter 1 – Introduction

Unidentified individuals

The increasing number of unidentified individuals has become a growing concern worldwide (Hanzlick & Smith, 2006; Kumar, Dasari & Singh, 2014; Reid, Martin & Heathfield, 2020; Sohn, Timmermans & Prickett, 2020). These individuals, whose identities remain unknown following medico-legal autopsy, present a complex challenge for authorities and communities alike. These unidentified bodies found in various locations add to the number of missing persons cases with no resolution, weighs heavily on law enforcement agencies, forensic experts, and families desperately seeking answers. Thus, the need for innovative investigative techniques, improved collaboration, and enhanced technology to tackle the mounting challenge of identifying these individuals and providing closure to their loved ones is necessary.

Globally, the percentage of unidentified individuals varies widely, ranging from less than 0.001% to as high as 24.4% (Reid, Martin & Heathfield, 2023). Developed countries typically have a lower number of unidentified individuals at the time of autopsy (with an average of 4.4%, ranging from less than 0.001% to 15.9%) compared to developing countries (with an average of 9.5%, ranging from 3.0% to 24.5%) (Reid, Martin & Heathfield, 2023). A study conducted in India over five years reported that 3.8% of mortuary admissions were unidentified individuals (Kumar, Dasari & Singh, 2014).

A systematic review of the prevalence of unidentified remains in the United States of America indicates a minimal rate of unidentified individuals in state mortuaries (Hanzlick & Smith, 2006; Sohn, Timmermans & Prickett, 2020). Following the systematic review, the authors found that only 0.2% of individuals in Georgia and 0.4% in California remained unidentified. In South Africa, specifically, Salt River Mortuary, Cape Town, had a rate of 9.2% unidentified individuals (Reid, Martin & Heathfield, 2020) between 1 January 2010 and 31 December 2017, and in Pretoria, South Africa the annual rate of unidentified individuals at state mortuaries was 9% (Evert, 2011). These findings highlight the number of unidentified individuals across South Africa. However, the data represented by these studies primarily focus on only a single mortuary, thus conclusions on identification rates at the national level cannot be made. It is also important to note that several other factors affect the number of unidentified individuals. This may include the methods used for identification, the rates of admissions to mortuaries and the laws surrounding the classification of unidentified individuals.

The results from studies investigating unidentified bodies give insight into different demographic groups. Many studies highlight that most of the individuals remaining unidentified after investigation are male (Baliso, Heathfield & Gibbon, 2022; Chattopadhyay, Shee & Sukul, 2013; Evert, 2011; Kaur et al., 2022; Kumar, Dasari & Singh, 2014; Reid, Martin & Heathfield, 2020; Reid, Martin & Heathfield, 2023). The most common age at death reported for each study varied, however a general overlap was noted. The study conducted in South Africa reported that most individuals were between the ages of 20-39 years (Reid, Martin & Heathfield, 2020); while in India, studies reported the most common ages for the reported unclaimed individuals to be 20-60 years of age (Kaur et al., 2022; Kumar, Dasari & Singh, 2014). These results highlight most unidentified individuals are adults.

Identification in South Africa

In the context of South Africa, forensic anthropology is not legislated, and most of the legislation regarding unidentified individuals has an emphasis on soft tissue (*National Health Act No. 61 of 2003*, 2003; *National Health Act No. 61 of 2003. Regulations: Rendering of forensic pathology services*, 2018). Thus, there are no set criteria or certification processes required to practice forensic anthropology (Steyn, L'Abbé & Myburgh, 2016). Most forensic anthropologists within South Africa are connected to tertiary education institutes. Forensic Anthropology Cape Town (FACT) is a forensic anthropology service that is based at the University of Cape Town (UCT). This laboratory assists in the identification of decomposed and skeletonized individuals in collaboration with Forensic Pathology Services (FPS) in the Western Cape. Baliso, Finaughty and Gibbon (2019) conducted a study highlighting the underutilization of forensic anthropology, using Salt River Mortuary in South Africa as an example. The research retrospectively examined cases investigated by FACT between 2008 and 2018, comparing it with the caseload of Salt River Mortuary during the same period. During this period, the mortuary received 345 (out of 23408) cases involving decomposed, burnt, or skeletonized remains. It is important to note that it is at the discretion of the pathologist whether a case is referred for anthropological analysis. According to FACT data, of the 345 cases involving decomposed, burnt, or skeletonized remains, only 73 cases were recommended for forensic anthropological analysis (Baliso, Finaughty & Gibbon, 2019).

Furthermore, a study conducted at Salt River Mortuary revealed that among the group of unidentified individuals examined, approximately 14.1% of cases were unable to be identified through physical means due to alterations to the body (Reid, Martin & Heathfield, 2020). Routinely, visual identification is sufficient, however, in cases where the decedent has sustained severe physical trauma or changes due to taphonomic conditions, it can make visual identification difficult.

Molecular and radiographic analyses, such as DNA fingerprinting or radiography, can be utilised in instances where visual identification is not applicable (National Health Act, Act No 61 of 2003; National Health Act No. 61 of 2003. Regulations: Rendering of forensic pathology services, 2018). Another avenue of identification includes anthropological techniques. Anthropological techniques also prove useful in the event of advanced decomposition and/or skeletonization, where primary means of identification are not usually possible. The skeletal remains/hard tissues of the decedent are used to estimate information regarding the identity and death of the individual by generating a biological profile. This consists of estimating demographic variables such as an individual's sex, age, possible population affinity and identifying factors of individualization including trauma and living antemortem height.

Forensic anthropology is an interdisciplinary field that has foundations in anthropology, skeletal biology and archaeology. Forensic anthropologists aim to answer four objectives (Byers, 2016). This includes generating a biological profile (which helps estimate demographic variables of the decedent); analysing the remains for any signs of trauma; estimating a probable post-mortem interval (PMI); and identifying any unique features present on skeletal elements can assist in positively identifying deceased individuals.

Sex estimation in forensic anthropology

Sex estimation is a crucial component pertaining to forensic anthropology when generating a biological profile. This aspect relies on sexual dimorphism to distinguish biological females and males. Sexual dimorphism refers to the variations between males and females within a species, encompassing distinctions in aspects such as body size, shape, developmental pace, and behaviour. This phenomenon arises from a combination of genetic factors (intrinsic factors), such as hormone levels, and environmental influences (extrinsic factors), including nutrition and cultural behaviours (Stinson, Bogin & O'Rourke, 2012). FACT routinely use non-metric methods when estimating sex for biological profiles (Baliso, Finaughty & Gibbon, 2019;

Baliso, Heathfield & Gibbon, 2022), as these methods are quick and easy to apply, as well as having widespread usage in the respective field.

These methods include examining the cranium and pelvis to estimate sex and to a lesser extent the long bones and overall robusticity of the skeleton (Buikstra and Ubelaker, 1994; Phenice, 1969 and Klales, Ousley and Vollner, 2012). Methods that make use of the cranium include those described in Ubelaker and Buikstra (1994), where bony landmarks such as the nuchal crest, mastoid process, supraorbital margin, glabella and mental eminence are examined. The Phenice (1969) method employs the pelvic bones, where the ventral arc, subpubic concavity and ischiopubic ramus are scrutinised (Klales, Ousley & Vollner, 2012; Ubelaker & Buikstra, 1994). A method validation study done by Klales, Ousley and Vollner (2012) of the Phenice (1969) method, indicated that the ventral arc proved to be the best trait for discrimination for females and males (with an 88.5% correct classification), followed by the subpubic contour (with an 86.6% correct classification) and lastly the ischio-pubic ramus (75.8% correct classification). A retrospective analysis of the cases consulted by FACT indicates that sex estimation was correct for individuals in all cases where positive identification was confirmed (Baliso, Finaughty & Gibbon, 2019). Despite these successful estimates, it is important to consider the pros and cons that accompany non-metric methods and compare them to those of metric methods.

Non-metric methods are quick to apply in the estimation of sex. However, Klales (2020) demonstrates that non-metric methods are prone to variable validity and reliability, as they lack objective scoring and a statistical approach. However, morphoscopic methods (e.g. Walker, 2008 and Klales et.al., 2012) which apply scoring of morphological characteristics are better than traditional non-metric methods. Furthermore, an important consideration is the population used to establish these morphological methods, as the same degree of variation may not exist in all populations. Klales (2020) went further to say that metric estimation performs better in terms of validity and reliability, however, does not have the ease and speed of morphological analysis. Metric analysis is less prone to intra- and inter-observer error while offering a more statistical approach that is rooted in mathematical evidence when identifying an individual (DiGangi & Moore, 2013). This can assist expert witnesses in giving testimony to the reliability and validity of their casework.

When considering metric analysis, it is important to note that greater interobserver variability can arise when using methods/ models that do not make use of well-defined landmarks and when the researcher does not have a lot of experience with osteometric measurements (Adams & Byrd, 2002). Metric and morphoscopic analysis also follows the criteria of methods required by the Daubert ruling which emphasises that methods used by expert witnesses should be a testable theory or technique, subject to peer review and publication, have a known error rate and have widespread acceptance in the relevant scientific community (Farrell, 1993).

Use of cancellous bone for sex and age estimation

Cancellous bone consists of a network of interconnecting horizonal and vertical rods-like and plates-like structures forming a porous cancellous structure. This trabeculae architecture allows for bone to withstand the compressive forces experienced during everyday locomotion such as walking and running. Sexual dimorphism has been observed in the cancellous structure of various bones in the body (Chen et al., 2010; Eckstein et al., 2007; Mosekilde, 1989; Riggs et al., 2004; Rowbotham et al., 2022). Studies focusing on sexual dimorphism in cancellous bone include bones such as the vertebral bodies (Eckstein et al., 2007; Mosekilde, 1989), neurocranial bones (Rowbotham et al., 2022) and the femur (Chen et al., 2010; Eckstein et al., 2007; Riggs et al., 2004), highlighting the potential avenues for further investigation of sexual dimorphism in different populations.

Mosekilde (1989) investigated the sex-related differences that accompany changes in the cancellous bone mass and structure in the vertebral body cancellous bone. This study highlighted that structural analysis demonstrated a steady age-related decline in the horizontal cancellous thickness that spans from medial to lateral dimensions in males and females. The mean cancellous thickness was higher for younger men than for younger women, however it was not statistically significantly greater. Vertical cancellous thickness showed no significant decrease in mean cancellous thickness for either males or females; however, the cancellous thickness for men showed a tendency to decrease with age. Females showed a small insignificant increase.

Furthermore, the age-related changes however did not differ significantly between males and females (Mosekilde, 1989). Linear regression analysis observing the distance between horizontal trabeculae (Reid, Martin & Heathfield, 2020) indicated that males displayed a steady increase in the distance between horizontal trabeculae, while females showed a slightly greater increase. With increasing age, males and females displayed an identical increase in distance between vertical trabeculae. Biomechanical stress testing of cancellous bone cylinders noted decreases in strength with increasing age. The rate of decrease in strength with age, were identical for both males and females; however, a pronounced decrease in strength was noted around the age of 40-50 for females in the horizontal cancellous cylinders. For the vertical cancellous bone cylinders, a decrease from 75-80% in strength was noted from the age of 20 to the age of 80 in both females and males. Similarly, the horizontal cancellous bone cylinders (cancellous spanning from medial to lateral) displayed a 90-95% decrease in strength from the age of 20 to the age of 80.

The study also examined the effects of menopause and advanced age on sex-related differences. This was done by subdividing the females into three different age groups (50 years, 50-75 years, and over 75 years) and comparing them to age-matched males (Mosekilde, 1989). The anisotropy index (the ratio of vertical compressive stress to horizontal compressive stress) indicated that females below the age of 50 had higher values compared to males. For individuals between the ages of 50-75 years, the anisotropy index was still higher than in males. The vertical cancellous thickness and the horizontal stress values were lower in females than in males. Females older than 75 years of age displayed a greater distance between horizontal trabeculae compared to the age-matched males. It has been shown that in menopausal women, normal bone turnover is affected (Ji & Yu, 2015). During menopause, there is a reduction in the levels of oestrogen in women. Oestrogen is responsible for inhibiting the reabsorption of bone done by osteoclasts. This results when there is an increase in osteoclast activity. The increased reabsorption coupled with age-related bone loss results in expedited loss of bone in females (Heersche, Bellows & Ishida, 1998; Ji & Yu, 2015). These changes in bone mass overall decrease the strength of bone and increase the risk of diseases such as osteoporosis.

Rowbotham et al. (2022) investigated the average density and thickness of neurocranial bones in an Australian population. Post-mortem computer tomography (PMCT) was used to measure density and thickness in 20 different regions of the neurocranium. The results of their investigation revealed that significant differences exist between males and females in various regions of the neurocranium in terms of density. These regions included all regions of the parietal bones that were observed, the occipital protuberance, left and right mid-occipitals, left temporal squama, left and right mastoids and petrous portion. Furthermore, the interaction between age and sex had a significant impact on the differences in all portions of the neurocranium except five of the twenty regions that were investigated. When observing the effects of age on the density in the different sexes, neurocranial density tended to increase with age in all regions (except that of the squamosal, mid-occipital and basi-occiput regions) in males, while in females the density tended to decrease with age. Rowbotham et al. (2022) focused on the flat bones of the skull, which develop through intermembranous ossification; while the current study focuses on the femur which is a long bone that develops through a combination of endochondral and intramembranous ossification (Clarke, 2008). It is important to note the composition of cortical to cancellous bone in the two sites as well, as it plays a role in the density of bone (Clarke, 2008; Sambrook, Kelly & Eisman, 1993). Osterhoff et al. (2016) note during the early process of osteoporosis, initially cancellous bone is lost; with increasing age, the porosity of the cortical bone then increases. These insights can elucidate some of the interpretations of the results of Rowbotham et al. (2022).

Sexual dimorphism in the femur

The femur makes up the part of the upper lower limb and consists of a layer of cortical bone on the outside, and cancellous bone on the inner sections of the bone. The femur has been shown to exhibit sexual dimorphism in different populations (Asala, SA, 2001; Chen et al., 2010; Eckstein et al., 2007; İşcan & Shihai, 1995; King, İşcan & Loth, 1998; Macho, 1990; Purkait, 2003; Riancho et al., 2007), making it an ideal location for investigating sexual dimorphism. A South African study investigating sex estimation in two different South African populations found population-specific sex differences (Asala, 2001). The data displayed significant mean differences between sexes in the mean vertical head diameter of the femora in the "South African white population"; while the mean transverse head diameter of the femora displayed significant differences between sexes in the "South African black population". This indicates how different parameters of the femur may be more beneficial in the estimation of sex in different population affinities. Similarly, Steyn and İşcan (1997) looked at femoral and tibial measurements in terms of sex estimation. It was found that both the distal bredths of the femur and tibia were the best discriminators. Asala, Bidmos and Dayal (2004) highlighted that vertical head diameter was the most discriminating part of the upper femur (as it had a correct classification percentage of 82.6%), while the bicondylar breadth was the most discriminating part of the lower femur (it has a correct classification percentage of when it came to sex estimation.

Krüger, L'Abbé and Stull (2017) looked at sex estimation from long bones in a modern South African population. Measurements were taken from post-cranial elements, with one being the femur. Eight standard femoral measurements including maximum femoral length, femoral bicondylar breadth, femoral vertical head diameter and subtrochanteric anterior posterior diameter. LDA and flexible discriminant analysis (FDA) were generated using various post-crania elements to produce both univariate and multivariate models. When the population affinity groups were pooled, both the LDA and FDA univariate models for the femora had a correct classification percentage ranging from 73%-86%. When looking at the multivariate models for the femur, the LDA had an average correct classification percentage of 86%, while the FDA had an average correct classification percentage of 87%. The authors highlight that the multivariate models performed better compared to the univariate models, as the models make use of most sexually dimorphic elements, providing more reliable results.

Riggs et al. (2004) investigated age-related changes in central and peripheral bone sites using quantitative computed tomography (QCT). These changes were compared between sexes and how they contribute to the difference in risk for fracture between elderly females and elderly males. Young adult females showed a cross-sectional area that was less than young adult males by 25-33% at central (the lumbar spine and proximal femur) and peripheral (distal radius and distal tibia) bone sites. A similar difference was noted with bone mass, which ranged from an 18-21% difference (Riggs et al., 2004). Cortical vBMD displayed considerable decreases in cortical bone at the femoral neck, as well as the peripheral scanning sites. These decreases were noted to be minimal around the midlife point in both females and males. Thereafter, a linear decrease was noted in both sexes, however, a greater decrease was noted in females (28%) compared to males (18%) (Riggs et al., 2004).

A study investigating three-dimensional microstructural changes of cortical and cancellous bone in the femur of a Japanese population showed that males had significantly higher levels of bone volume/total volume (BV/TV) ratios compared to females (Chen et al., 2010). Males also displayed a lower expression of cancellous separation; while also maintaining higher cancellous thickness measurements, cancellous numbers and connectivity density. DiBennardo and Taylor (1979) investigated the gross morphology of the femur for sexual dimorphism through a series of measurements (i.e., maximum femoral length from the femoral head to the medial condyle, and three measurements at mid-shaft: circumference, maximum anteroposterior diameter, and transverse diameter) which were used to generate discriminant function analyses. Their results indicated that using the circumference alone gave as good an estimate as that of the accuracy of any multiple discriminant function in sexing the femur (DiBennardo & Taylor, 1979). The univariate functions gave an estimate range of 80-83%, while multivariate functions gave estimate ranges of 79-86%.

A German population study investigated how sex and age affect cancellous microstructure using computed tomography (CT) scanning in different sites (such as the pelvis, proximal femur, calcaneus, distal radius, and second lumbar vertebra) (Eckstein et al., 2007). The findings indicated that the BV/TV ratio was highest in the femoral neck for males and highest in the calcaneus for females. They also noted that females displayed significantly lower BV/TV ratios at the distal radius, femoral neck and trochanter. Furthermore, cancellous thickness was shown to have significant sex-related differences at the distal radius, femoral neck and trochanter. Males displayed the highest degree of trabeculae separation and the lowest number of trabeculae at the iliac crest, while females displayed this at the femoral neck (Eckstein et al., 2007). A Thai population study investigating sexual dimorphism in the femur (King, İşcan & Loth, 1998) revealed measurements of the parameters investigated (Maximum length, Vertical head diameter, Midshaft circumference, Midshaft anteroposterior diameter, Midshaft transverse diameter, Bicondylar breadth) were greater for men overall compared to females. They also concluded that the maximum head diameter and bicondylar breadth were the most optimal combination for sex estimation (King, İşcan & Loth, 1998).

Riancho et al. (2007) observed the effects of age and sex on biomechanical indices in the femoral neck within a Spanish population. The results of their study indicated that within the younger individuals, males had higher bone mineral densities compared to females. The ratio of bone mineral content to the area of the femoral neck decreased in both sexes; however, a greater loss was noted in females. This finding was similar in the volumetric bone mineral density, where a decrease was seen in both sexes; however, the decrease occurred more rapidly in women. Average cortical thickness in the femoral neck in younger individuals was shown to be greater in males, and with age, cortical thickness tended to decrease more rapidly in females. The buckling ratio (which was defined as an index of the local cortical instability that increases when the ratio of the outer radius to the wall thickness of a thin tube exceeds a certain value) increased with age in both sexes but the slope was higher in women (Riancho et al., 2007).

These studies highlight that traits that display sexual dimorphism vary greatly across populations as different populations experience different environmental pressures, different gene pools and different regional secular trends (Ubelaker & DeGaglia, 2017). To this extent, the literature suggests that morphological traits and methods developed for specific populations should be utilized. Ideally, these population-specific developed methods should be compared on different population cohorts to illustrate whether methods developed on non-reference cohorts are applicable (Ubelaker & DeGaglia, 2017).

Anthropological studies that focus on bone measurements can either take a 2-D or 3-D approach. Traditional radiography produces scans of bones in 2-D, while some imaging modalities such as CT produce 3-D scans of bones. The current study takes a 2-D approach, whereby measurements will be taken on dry bone, as well as on radiographs generated from a LODOX instrument. While a 3-D approach can be adopted by using the measurements extracted from radiographs generated and used to generate a 3-D model of bones, that is beyond the scope of this study.

The use of radiography in forensic anthropology

Research on anthropological analysis using radiographs has been extensively documented (Franklin, Swift & Flavel, 2016; Mamabolo, Alblas & Brits, 2020). Studies such as Lynnerup, Thomsen and Frohlich (1990) use non-invasive techniques for age estimation utilizing the proximal femur, which revealed correlations between changes in cancellous bone and individuals' ages, particularly in living individuals with intact soft tissue. Walker and Lovejoy (1985) investigated radiographic changes in the clavicle to understand bone involution's correlation with age, highlighting the clavicle's consistent relationship with age in both sexes, especially in males.

Similarly, Macchiarelli and Bondioli (1994) explored cancellous bone patterns in the proximal femur, noting a more pronounced decrease in bone density in females and observing site- and sex-specific patterns of cancellous bone loss. The radiographs used in this study made use of two different image processes, digital image processing (DIP) and laser linear densitometric analyses (LLDA) (Magat & Ozcan Sener, 2019). Their study also revealed moderate linear correlations between cancellous bone characteristics and age.

de Froidmont et al. (2013) evaluated the reliability of multi-detector computed tomography (MDCT) and conventional X-rays for long bone cancellous analysis. Their study assessed cancellous destruction in the proximal femur and humerus, employing a schema described by Nemeskéri, Harsányi and Acsädi (1960). The research highlighted the greater reliability of femoral head measurements using conventional X-rays and the greater reliability of MDCT for humeral head measurements. Interobserver reliability did not significantly differ between MDCT modalities, and MDCT generally exhibited higher reliability than conventional X-rays, particularly regarding the Nemeskéri, Harsányi and Acsädi (1960) schema. Correlations between measurements were notable, particularly between MDCT modalities, indicating the potential of MDCT for accurate anthropological analysis.

Fractal Analysis

Fractal analysis (FA) is a quantitative method to evaluate bone volume and architecture (Lopes & Betrouni, 2009). FA allows for complex patterns to be detected in the microarchitecture of the bone and assign a numerical value. The research into fractal analysis highlights the potential it has in evaluating cancellous bone differences, however, research regarding the utility of FA to assess sexual dimorphism in post-cranial elements has not been expressly considered. The use of fractal analysis for sexual dimorphism within the mandible has been explored by dos Santos Menezes et al. (2021) and Santos et al. (2023). dos Santos Menezes et al. (2021) made use of plain film radiographs and found no significant difference between females and males; while Santos et al. (2023) opted for CT and found a significant difference between females and males.

Furthermore, studies utilizing fractal analysis have highlighted a correlation between fractal dimensions and biomechanical properties (Grampp et al., 1999; Sanchez-Molina et al., 2013). Grampp et al. (1999) aimed to assess the comparison between biomechanical factors (such as maximum compressive strength (MCS) and maximum sheer stress (MSS) and fractal dimensions. Their study found that MCS and MSS both had a significant correlation with fractal dimension. Furthermore, Sanchez-Molina et al. (2013) investigated the correlation between the fractal dimension and biomechanical properties such as failure stress and Young's Modulus (which describes the tensile elasticity of a material) of cortical bone of the ribs. The results of the study highlighted the statistically significant correlation between both biomechanical properties and fractal dimensions.

The Singh Index

The Singh index is a screening tool, which assigns a score to a radiograph which informs apparent bone density and the architecture of the cancellous network (i.e., it gives an indication of the state of the cancellous bone density) (Singh, Nagrath & Maini, 1970). Limited research focuses on whether the Singh index can be used as a tool for sex estimation and whether it can be incorporated into the generation of a biological profile. The Singh index can likely be used when looking at specific age categories during sex estimation as Tsangari, Findlay and Fazzalari (2007) suggested that mechanisms leading to structural changes in cancellous bone are different for females and males.

Studies investigating reproducibility of the Singh Index have found mixed results. A study done by Koot, Kesselaer and Clevers (1996) looked at 80 different radiographs of the proximal femur. One of their aims was to validate and observe the rate of agreement between observers using the Singh index between six different observers (with varying levels of experience). The intra-observer agreement indicated promising values (Kappa ranging from 0.63 to 0.88), however, there was poor interobserver agreement (Kappa ranging from 0.15 to 0.54). Their second aim was to investigate the correlation between bone mineral density and the Singh index. The bone mineral densities were measured using dual-energy x-ray absorptiometry (DEXA) and ranged from 0.35 g/cm^2 to 1.14 g/cm^2. They concluded that there was no correlation between BMD and the Singh index.

Wachter et al. (2001) conducted a study, where they investigated the predictive value of the Singh index and BMD for cancellous bone quality in the intertrochanteric region. Their study found good levels of intra-observer agreement (67%) and interobserver agreement (63%). This study looked at the mechanical properties of bone biopsies, taken from the intertrochanteric region, such as elastic modulus, bone strength and maximum energy absorption. All three parameters showed strong correlations with the Singh index (with r values ranging from 0.66-0.73), with bone strength being the highest (0.73). The authors noted a significant correlation between the Singh index and local mechanical cancellous bone properties of the intertrochanteric region measured.

Similar results were found by Masud et al. (1995), who assessed the usefulness of the Singh index by comparing it to the gold standard of DEXA. This study compared the bone mineral densities generated by DEXA, of bone cores biopsied from the femoral neck to that of the scores of the Singh index. The intra-observer agreement using the Kappa statistic was 0.64, while the interobserver agreement kappa statistic was 0.63.

Limited research focuses on whether the Singh index can be used as a tool for sex estimation and whether it can be incorporated into the generation of a biological profile. The Singh index can likely be used when looking at specific age categories during sex estimation as Tsangari, Findlay and Fazzalari (2007) suggested that mechanisms leading to structural changes in cancellous bone are different for females and males.

Rationale

The number of unidentified remains particularly in South Africa is evident and this is expected to increase with an increasing population size and a rise in migratory workers (Crush, Chikanda & Tawodzera, 2015; Mlambo, 2018). Forensic anthropology is a valuable tool to assist in the identification process, however further research is necessary to alleviate the increasing number of unidentified individuals. As research has shown, imaging techniques such as X-rays and CT scanning have become an avenue in which research into the field of anthropology has expanded. Imaging techniques allow for research on internal structures of bone to be done without invasive methods needing to be used. LODOX imaging finds itself in an ideal position due to its widespread availability in most mortuaries in South Africa, primarily used for trauma analysis and identification of foreign objects (Knobel, Flash & Bowie, 2006); furthermore, it is used for purposes such as identification and anthropological analysis (Morele, Hill & Keyes, 2024). Further research needs to be done using LODOX imaging to establish how it can be used in a forensic anthropology setting. While cancellous bone assessment and radiography for sex & age estimation, as well as the effects of age progression on both cancellous and cortical bone, have been previously explored, the utility of alternative measures of bone density in the femur such as fractal analysis and the Singh index has been under-explored.

Aims and objectives

The overall aim of the study is to assess radiographic and dry bone measurements of the proximal femur for sex estimation in a South African population. This was achieved through the following objectives:

- Assess various radiographic parameters for association with sex.
- Assess various dry bone parameters for association with sex
- Utilize logistic regression to assess the best combination of variables for sex estimation.
- Assess the applicability of logistic models in a forensic cohort.

Chapter 2 - Materials and methods

Ethical approval

Ethical approval for this study was obtained from the Human Research Ethics Committee (HREC) of the University of Cape Town (UCT) (Ref No. 381/2023). Approval from the UCT Human Skeletal Repository at the University was also received to use individuals in the UCT Skeletal Repository (as seen in Appendix A).

Sample selection and inclusion/exclusion criteria

The study consisted of two cohorts (cadaveric and forensic); however, the same selection criteria were applied to both. The cadaveric cohort consists of individuals with known sex and age from individuals who have donated their bodies to the Department of Human Biology at UCT for use in scientific or medical research. The forensic cohort consists of unidentified skeletal remains recovered by the South African Police Service and the Forensic Pathology Services and are used for teaching and research purposes following permission from the Inspectorate of Anatomy. Individuals were included in the study if:

- The individual had at least one femur present.
- The femur was free from any alteration to the extent where the cancellous bone was affected.
- The individual was an adult (individuals who are 20 years and older)

The inclusion criteria were applied to the human remains in the Skeletal Human Repository at UCT. This resulted in the final sample size for the cadaveric cohort being 90 individuals, while the final sample size for the forensic sample consisted of 30 individuals.

As the forensic cohort consists of unknown individuals, the estimates generated by FACT and other independent researchers, such as sex and age, were used to select individuals from the cohort (Baliso, Heathfield & Gibbon, 2022). Sex estimates were determined using methods that make use of the cranium and pelvis (Klales, Ousley & Vollner, 2012; Phenice, 1969 ; Ubelaker & Buikstra, 1994); while the methods used for age estimation included using the pubic symphyseal surface of the pubic bone, cranial suture closure, the use of sternal rib ends, examination of the iliac auricular surface, dental eruption/formation and epiphyseal fusion pelvis (Buckberry & Chamberlain, 2002; Esan & Schepartz, 2018; Iscan, Loth &

Wright, 1993; Işcan, Loth & Wright, 1984; İşcan & Shihai, 1995; Schaefer et al., 2009; Skinner, 1989; Ubelaker & Buikstra, 1994). These estimates were used to exclude any juveniles from the study.

Dry bone measurements

Ideally, the left femur of each individual was assessed, however, if the left femur was not present, the right was selected. A series of femoral measurements (Table 1) were obtained on dry bone with the use of an osteometric board and a digital sliding calliper (both instruments have been calibrated). These measurements were selected for comparative purposes, as they will be compared to those taken on the radiographs in cases of the Anterior-Posterior Thickness, Femoral Head Diameter and the length Greater trochanter to the fovea capitis (GT – FC). The inclusion of the Maximum Length of the Femur and the Physiological Length of the Femur was done as these measurements have been shown to have discriminative power when it comes to sex estimation.

Table 1: Dry bone measurements recorded from each femur in the study.

Measurement	Description	Reference
Maximum Length of the Femur	The length of the femur from the femoral head to the medial condyle of the femur.	Ubelaker & Buikstra (1994)
Physiological Length of the Femur	The length of the femur from the femoral head to distal end with both condyles of the femur touching the osteometric board.	Ubelaker & Buikstra (1994)
Anterior-Posterior width	Length of the anterior-posterior thickness of the femur at the level of the lesser trochanter.	İşcan & Shihai (1995)
Femoral Head Diameter	The diameter of the femoral head.	Ubelaker & Buikstra (1994)
The length Greater trochanter to the fovea capitis (GT – FC)	The most lateral apex on the greater trochanter to the superior margin on the fovea capitis.	Albanese, Eklics & Tuck (2008)

Radiographic assessment

Digital radiographs of each femur were produced in the anteroposterior (AP) and mediolateral (Mlambo) plane with the use of an EXERO/DR LODOX instrument (Figure 1). Scans were performed at 70 kV and 80 mA, with an exposure time of 44 msec. Bones were fixed in place in the required orientation when scanning. All scans were conducted at the Observatory Forensic Pathology Institute.

Digital radiographs were exported in both DICOM and JPEG format for further radiographic assessment. Digital Radiographs were imported into OsiriX (Pixmeo SARL, version 12.0) that was utilised for linear measurements. The length function under the region of interest tool was used for this purpose. Measurements were taken on both the AP and ML radiographs (Table 2).

The external and internal femoral neck measurements were used to establish the torsional and bending strength of each femur at the femoral neck. The torsional and bending strength in the context of the femoral neck refers to the ability of the femoral neck to withstand twisting forces being applied, while the bending strength refers to the femoral neck's ability to withstand bending forces being applied.

The equation used for estimating bending strength (Hearn, 1985):

$$= \frac{\pi((external\ neck\ diamter^{4}) - (internal\ neck\ diamter^{4}))}{64}$$

The equation used for estimating tortional strength (Hearn, 1985):

$$= \frac{\pi((external\ neck\ diamter^{4}) - (internal\ neck\ diamter^{4}))}{32}$$

Table 2: Radiographic measurements recorded from each femur in the study.

Measurement (scan taken on)	Orientation	Description	Reference
Anterior-Posterior width	ML	Length of the anterior-posterior thickness of the femur at the level of the lesser trochanter.	İşcan & Shihai (1995)
Femoral Head Diameter	AP	The diameter of the femoral head.	Ubelaker & Buikstra (1994)
Length of the Proximal Femur	AP	The furthest point of the proximal femur from the femoral head to the distal base of the lesser trochanter.	Albanese, Eklics & Tuck (2008)
Medial Cortical Thickness	AP	The length of the cortical bone on the proximal femur, inferior to the lesser trochanter on the medial side of the proximal femur.	Chen et al. (2010)
Lateral Cortical Thickness	AP	The length of the cortical bone on the proximal femur, inferior to the lesser trochanter on the lateral side of the proximal femur.	Chen et al. (2010)
Anterior Cortical Thickness	ML	The length of the cortical bone on the proximal femur, inferior to the lesser trochanter on the anterior side of proximal femur.	Chen et al. (2010)
Posterior Cortical Thickness	ML	The length of the cortical bone on the proximal femur, inferior to the lesser trochanter on the posterior side of the proximal femur.	Chen et al. (2010)
External Femoral Neck Diameter	AP	Midpoint on the femoral neck at the proximal end to the midpoint of the femoral neck at the distal end on the AP scan	Hearn (1985); Riancho et al. (2007)
Internal Femoral Neck Diameter	AP	Midpoint on the femoral neck at the proximal end to the midpoint of the femoral neck at the distal end of the internal cancellous bone on the AP scan	Hearn (1985); Riancho et al. (2007)

Key: AP - anteroposterior; ML - mediolateral

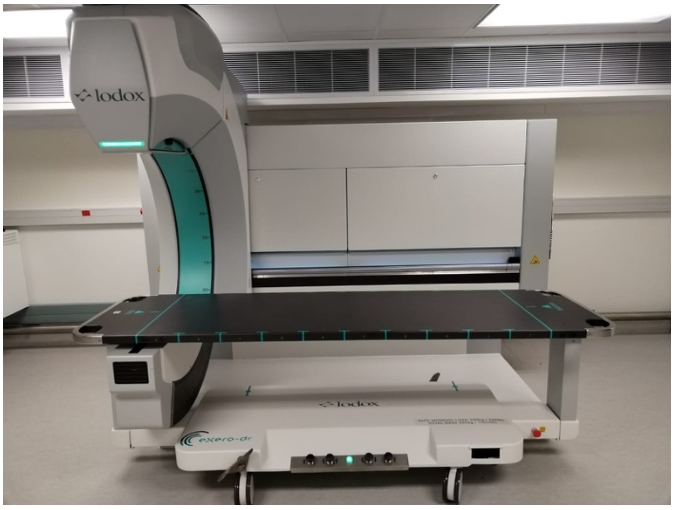

Figure 1: EXERO/DR LODOX instrument.

Fractal Analysis

Fractal analysis (FA) has been shown to be a quantitative method to evaluate bone volume and architecture (Lopes & Betrouni, 2009), yet its application in forensic anthropology has been under-investigated. FA allows for complex patterns to be detected in the microarchitecture of the bone and assign a numerical value. This value is known as the fractal dimension and is an expression of bone structural complexity. Research into fractal analysis focuses mainly on changes in the bone of the mandible (Hayek et al., 2020; Soltani et al., 2021). Fractal analysis for the use of forensics has been conducted and extends into post-mortem changes (De-Giorgio et al., 2022), age estimation at death (Kucheryavski, Belyaev & Fominykh, 2009; Obert et al., 2014; Obert et al., 2017) and antemortem ageing (Marzi et al., 2020).

Fractal analysis was used to assess the cancellous content and architecture of the femora. The fractal dimension observes the complex repeating structural patterns of an object and generates a value based on the complexity of the structure (Huh et al., 2011; Yasar & Akgunlu, 2005). The more repeating units in a space, the greater the complexity and the higher the value. In the context of bone tissue, this provides information regarding the volume of bone.

Fractal analysis was conducted using Fiji software (ImageJ version 2.14.0) utilising the BoneJ plugin (Doube et al., 2010) following the method described by Magat and Ozcan Sener (2019) with adaption as described below. AP radiographs were imported to Fiji and the scale was set before fractal analysis commenced. The regions of interest (ROIs) were located, and the image was cropped into 1x1 cm blocks. These ROIs were selected based on the pattern and density of the trabeculae to look at the density of trabeculae at sites of interest as seen in Figure 2 Panel A (Singh, Nagrath & Maini, 1970). The three regions of interest (Figure 2B) included were: (1) the intersection of the primary and tensile compressive trabeculae (Figure 2A and 2B, green box), (2) the area above Ward's triangle where the primary tensile trabeculae run along (Figure 2A and 2B, blue box), (3) the intersection of the primary tensile and secondary compressive trabeculae (Figure 2A and 2B, purple box).

The cropped ROI was then duplicated. To reduce the amount of artefacts/noise of the radiograph, a Gaussian blur was added to the duplicated image with a sigma value of 5. The image calculator function was then used to subtract the edited duplicate from the original duplicate. The resultant image was then converted to a binary image and thereafter skeletonized. The fractal dimension function in BoneJ under the plugin function is then used. The automatic parameter function was then selected to generate the fractal dimension functions.

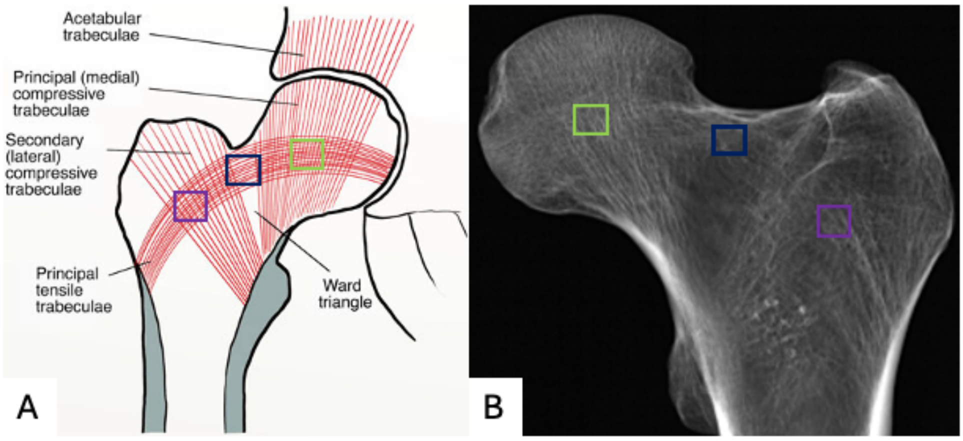

Figure 2: Panel A: The different trabeculae groups found in the proximal femur. Image adapted from (Kanakaris & Lasanianos, 2014); Panel B: The three different ROIs used for Fractal Analysis. Green box/ROI 1: intersection of the primary and tensile compression trabeculae. Blue box/ ROI 2: area above Ward's triangle where the primary tensile trabeculae run. Purple box/ ROI 3: intersection of the primary tensile and secondary compressive trabeculae.

Singh Index

The Singh index is a screening tool, which assigns a score to a radiograph which informs apparent bone density and the architecture of the cancellous network (Singh, Nagrath & Maini, 1970). The index ranges from a score of one to six and looks at the cancellous subgroups (i.e., the principal compressive group, the principal tensile group and the secondary tensile group). Higher scores indicate more visible cancellous groups compared to lower scores, where there is a gradual fade in the visibility of the cancellous groups. This index is usually used in combination when evaluating bone mineral density for diagnosis of osteoporosis, as in isolation, it is not considered a viable tool to evaluate the mechanical competence of bone (Alabdah et al., 2023).

The cancellous architecture was graded using the Singh Index. Radiographs were scored using a diagram adapted from Kanakaris and Lasanianos (2014) (Figure 3). The radiographs are scored based on the grading proposed by Singh et al (1970).

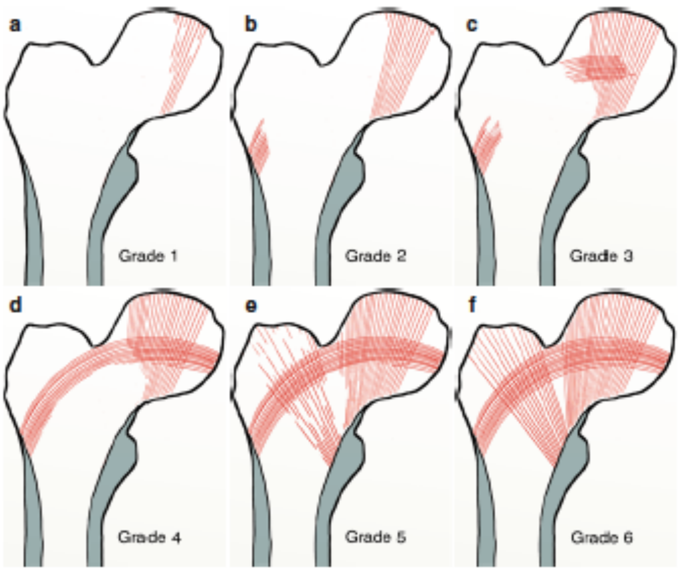

Figure 3: The different grades in the Singh Index (Singh, Nagrath & Maini, 1970). Image adapted from (Kanakaris & Lasanianos, 2014).

The index grades the cancellous content of the femoral head and neck by examining the presence and magnitude of three different trabeculae groups (Figure 2). Firstly, it looks at the principal tensile trabeculae which span horizontally from the femoral head to the trochanteric region. Secondly, it looks at the principal (medial) compressive trabeculae, which span vertically from the femoral head to the lesser trochanter. Finally, it looks at the secondary (lateral) compressive trabeculae, which span vertically from the greater trochanter to the lesser trochanter.

Femoral head and neck area

Area measurements of the femoral head and neck in each femur were assessed in the Fiji software (ImageJ version 2.14.0). The areas recorded included the cortical area of the femoral head and neck, as well as the area of the cancellous bone of the femoral head and neck. These measurements were conducted on the proximal femur in the AP plane. In the program Fiji, the radiographic image was cropped to the femoral head and neck region, inferior to the lesser trochanter (Figure 4A). The total area was generated by using the sliders in the threshold function (Image>Adjust>threshold). The area was then selected using the wand tool and measured. The image was then reset (the threshold function is set back to normal). The brush tool was then used to outline the cancellous bone (a brush width of 2 pixels was used). The outline of the cancellous bone is then selected using the wand tool and measured. The cancellous area of the bone was then subtracted from the total area to get the area of the cortical layer of the femora. The cortical and cancellous area measurements were displayed in a percentage relative to the total area.

Figure 4: Process for area analysis. Panel A: ROI of the proximal femur. Panel B: the total area that is selected using the threshold function. Panel C: highlight of the cancellous area being measured.

Statistical analysis

Data was collated and stored in a Microsoft Excel spreadsheet. Statistical analysis was primarily conducted using SPSS Ver 29.0.0.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean, mode and standard deviation) were calculated in Microsoft Excel. All sub-sets regression was performed in Stata 13 (StataCorp LLC, College Station, TX, USA) to determine the best combination of parameters to be used to generate logistic regression models.

Intra- & Inter-rater measurements were conducted on a subset of 30 individuals from the cadaveric cohort. Intra and inter-rater reliability of numerical data was assessed using the intraclass correlation coefficient (ICC). A two-way mixed effects model was used to evaluate intra-rater observation, while inter-rater agreement was evaluated by using a two-way random effects model. To evaluate the intra- and inter-rater of the Singh index, unweighted Cohen's Kappa was used. Comparisons were conducted between dry bone and scan measurements. Paired t-tests were conducted to assess systematic differences in the measurement of anteroposterior length of the proximal femur, the diameter of the femoral head and the GT-FC between modalities. The technical error of measurement (TEM) was calculated for comparative analysis of the dry bone vs scan measurements.

To assess systematic differences between males and females, traditional hypothesis testing was employed. The assumption of Normality was assessed using the Shapiro-Wilk test and homoscedasticity (equality of variances) was assessed using Levene's tests. Independent t-tests (for data that met the normality and equal variance assumptions), Mann-Whitney U tests or Pearson's chi-square tests (for categorical data) were carried out to assess differences between females and males. The level of significance was set at a p-value < 0.05 for all statistical tests.

To determine variables which may be useful in predictive models of sex, crude binary logistic regression was performed. With this, a pseudo-R^2 value will be generated. This value indicates a measure of variability accounted for by the model Variables that had a significance value of ≤ 0.05 and a correct classification percentage of 70% were selected for inclusion into a larger model. To determine the best combination of variables, an all-subset regression was performed in Stata 13 (StataCorp LLC, College Station, TX, USA) utilizing the gvselect user-written model (Lindsey & Sheather, 2015). Stata 13 evaluates the models according to Akaike's information criterion (AIC) and Bayesian information criterion (BIC). These information criteria are methods used to evaluate models based on making accurate predictions and simplicity of the model, while also considering different additional criteria. However, they are similar in terms of the lower the AIC/BIC criterion values, the more ideal the model is (Lindsey & Sheather, 2015). The models with the best AIC and BIC criterion value were selected. Assumptions for logistic regression, including the normality of the residuals were assessed using Shapiro-Wilk testing. The chosen models were then rerun in SPSS to confirm the model's appropriateness and classification power. A sensitivity vs selectivity plot was generated to determine the appropriate sectioning-point for the selected models. The sectioning-point used is a value which is used to assign the classification of the individual.

Chapter 3 - Results

Reliability

The unweighted Cohen's Kappa value used to evaluate intra-rater observations of the Singh index was 0.353 (indicating a fair agreement) with a p-value of < 0.001, while the Kappa value for the inter-rater amounted to -0.017 (indicates that agreement is less than random chance) and a p-value of 0.708.

Results of the intra-class correlation coefficients can be found in Table 3. The lateral cortical thickness, posterior cortical thickness, the fractal dimension of the femoral head the cortical area, percentage of the cortical area to the total area as well as the percentage of the cancellous area to the total area showed moderate reliability ($0.5 \leq ICC < 0.75$). Anterior-posterior width (dry bone), the femoral head diameter (dry bone), medial cortical thickness, anterior cortical thickness, external femoral neck diameter, internal femoral neck diameter, bending strength, torsional strength, the GT-FC (radiograph), Anterior-posterior width (radiograph), the fractal dimension of the femoral neck, fractal dimension of the trochanteric region, the total area and the cancellous area showed good reliability ($0.75 \leq ICC \leq 0.99$). The maximum length of the femur, the physiological length of the femur and the GT-FC (dry bone) showed perfect agreement (ICC = 1).

Table 3: ICC values generated for the intra- and inter-rater reliability analysis.

Parameter	ICC (Intra)	ICC (Inter)
Maximum Length of the Femur	1	1
Physiological Length of the Femur	1	1
GT-FC (dry bone)	1	0.98
GT-FC (radiograph)	0.99	0.88
Femoral Head Diameter (dry bone)	0.99	0.99
Anterior-posterior width (radiograph)	0.97	0.92
Anterior-posterior width (dry bone)	0.93	0.92
Femoral Head Diameter (radiograph)	0.93	0.94
External Neck Diameter	0.92	0.93

Fractal Dimension of Femoral Neck (ROI 2)	0.91	0.48
Anterior Cortical Thickness	0.90	0.84
Internal Neck Diameter	0.90	0.87
Medial Cortical Thickness	0.87	0.69
Total Area	0.85	0.85
Fractal Dimension of Trochanteric Region (ROI 3)	0.84	0.46
Cancellous Area	0.83	0.79
Lateral Cortical Thickness	0.79	0.80
Cortical Area	0.79	0.80
Posterior Cortical Thickness	0.74	0.80
Fractal Dimension of Femoral Head (ROI 1)	0.72	0.39
Percentage Cancellous to Total Area	0.70	0.67
Percentage Cortical to Total Area	0.68	0.44

For the inter-rater relatability, the fractal dimension of the femoral neck (ROI 2), the fractal dimension of the trochanteric region (ROI 3), the fractal dimension of femoral head area (ROI 1) and percentage cortical to total area showed weak reliability (ICC<0.5). The medial cortical thickness and posterior cortical thickness showed moderate reliability ($0.5 \leq$ ICC < 0.75). The maximum length of the femur, the physiological length of the femur, the femoral head diameter (dry bone), the length proximal femur (dry bone), the femoral head diameter (radiograph), the external femoral neck diameter, the anterior-posterior width (radiograph), the anterior-posterior width (dry bone), the GT-FC (radiograph), the internal femoral neck diameter, the total area, the anterior cortical thickness, the lateral cortical thickness, the bending strength, the torsional strength, the cancellous area and percentage cancellous to total area showed good reliability ($0.75 \leq$ ICC ≤ 0.99). The maximum length of the femur and the physiological length of the femur showed perfect agreement (ICC = 1).

The comparison testing results between dry bone and radiograph measurements of identical parameters indicated that, on average, the anterior-posterior width and the GT-FC exhibited larger measurements in radiographs, whereas the femoral head diameter showed a larger measurement value in dry bone. Significant difference testing between the means of dry bone and radiograph measurements revealed significant disparities across all variables. The

TEM was also calculated for each variable. These measurement results are summarized below in Table 4.

Table 4: Mean (± sd) measurements of dry and radiographic bone within the cadaveric cohort, along with the results of significance testing conducted between these two modalities.

Variable	Dry Bone	Radiograph	p-value	TEM	%TEM
Anterior-posterior width (mm)	34.45 ± 4.01	37.94 ± 4.90	<0.001	0.47	13.08
Femoral Head Diameter (mm)	44.00 ± 3.57	43.11 ± 3.84	<0.001	0.18	4.05
GT-FC (mm)	96.12 ± 28.75	98.22 ± 9.34	<0.001	0.58	6.07

Cohort descriptions

A summary of measurements obtained from the cadaveric and forensic cohorts can be found in Table 5. A summary of the Singh index scores is shown in Figure 4. The cadaveric cohort consisted of 90 individuals (58 males and 32 females). The age range for the sample was 22 years to 61 years, with an average age of 47.93 years (± 10.84 years). The forensic cohort consisted of 30 adult individuals, with a total estimate of 15 males, 2 females and 13 individuals whose sex was indeterminant. The mode for the Singh Index of the cadaveric cohort was 5, while the mode for the forensic cohort was 4.

Table 5: Mean and standard deviations for each variable within the cadaveric and forensic cohorts.

Parameter (measurement unit)	Cadaveric Cohort (mean ± standard deviation)	Forensic Cohort (mean ± standard deviation)
Age (years)	47.93 ± 10.84	------ *
Maximum Length of the Femur (mm)	448.23 ± 26.17	453.68 ± 27.64
Physiological Length of the Femur (mm)	444.98 ± 26.31	450.05 ± 28.10

Anterior-posterior width (dry bone) (mm)	34.45 ± 4.01	34.14 ± 4.03
Femoral Head Diameter (dry bone) (mm)	44.00 ± 3.57	44.47 ± 3.10
Length Proximal Femur (dry bone) (mm)	96.12 ± 28.75	93.92 ± 7.30
Medial Cortical Thickness (mm)	51.90 ± 16.88	65.65 ± 18.69
Lateral Cortical Thickness (mm)	50.70 ± 11.24	66.46 ± 11.19
Anterior Cortical Thickness (mm)	39.70 ± 11.93	56.02 ± 17.89
Posterior Cortical Thickness (mm)	31.49 ± 13.77	40.86 ± 18.09
External Neck Diameter (mm)	35.15 ± 3.83	36.77 ± 3.29
Internal Neck Diameter (mm)	30.86 ± 3.91	31.85 ± 3.74
Bending Strength (mm^4)	31.42 ± 14.51	39.33 ± 13.17
Tortional Strength (mm^4)	62.85 ± 29.02	78.67 ± 26.33
GT-FC (radiograph) (mm)	98.22 ± 9.34	111.32 ± 9.53
Femoral Head Diameter (radiograph) (mm)	43.11 ± 3.84	44.18 ± 3.68
Anterior-posterior width (radiograph) (mm)	37.94 ± 4.90	43.31 ± 5.61
Fractal Dimension of Femoral Head (ROI 1) (FD)	1.50 ± 0.09	1.43 ± 0.08
Fractal Dimension of Femoral Neck (ROI 2) (FD)	1.42 ± 0.11	1.40 ± 0.08
Fractal Dimension of Trochanteric Region (ROI 3) (FD)	1.49 ± 0.09	1.46 ± 0.07
Total Area (mm^2)	505.91 ± 84.59	596.55 ± 90.41
Cortical Area (mm^2)	65.90 ± 17.55	84.04 ± 18.09

Cancellous Area (mm^2)	440.00 ± 76.66	512.52 ± 76.55
Percentage Cortical to Total Area (%)	15.18 ± 4.15	16.39 ± 2.45
Percentage Cancellous to Total Area (%)	86.92 ± 2.93	85.94 ± 1.80

* - no age as the cohort is unidentified.

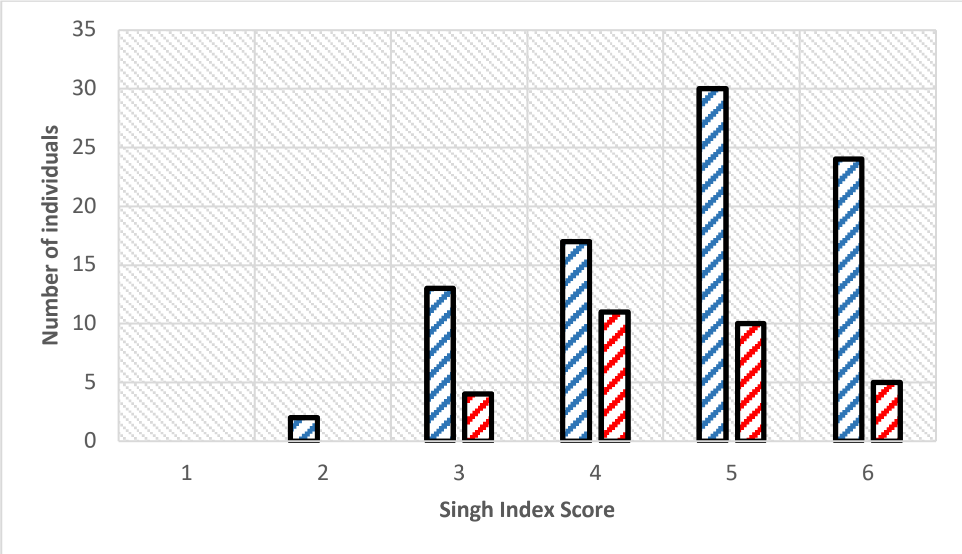

Figure 5: Singh Index scores in both cohorts. The Blue bars indicate the Singh Index scores for the cadaveric cohort, while the red bars indicate the Singh Index scores for the forensic cohort.

Sexual dimorphism in the cadaveric cohort

The age range for females spanned from 24 years old to 61 years with an average of 46.69 (± 11.06) years. The age range for males spanned from 22 years to 61 years with an average of 48.62 (± 10.76) years. The average age between males and females was not statistically significant ($p = 0.369$). A summary of the differences for each variable between males and females can be seen in Table 6.

The variables that were not normally distributed included: GT-FC (dry bone); Posterior Cortical Thickness; Bending Strength; Tortional Strength; GT-FC (scan); Anterior-Posterior width (scan); Fractal Analysis of Femoral Neck; Fractal Analysis of Trochanteric Region;

External Area; Percentage External to Total Area and Percentage Internal to Total Area. On average males had significantly larger measurements/results for the variables except the posterior cortical thickness, fractal dimensions of the femoral head (ROI 1), fractal dimensions of the trochanteric region (ROI 3) and percentage of cortical to total bone area. However, the posterior cortical thickness, fractal dimensions (femoral head and trochanteric region) and were not observed to be significantly different between the sexes. Females had a significantly larger percentage of cortical to total bone area (p = 0.017). Chi-square tests indicated no significant differences in the Singh index between females and males (p = 0.283). A graphical representation of some selected variables can be found in Figure 7.

Table 6: Averages and standard deviations for females and males, along with the results of significance testing.

Variable (SI unit)	Females	Males	p-value
Age (years)	46.69 ± 11.06	48.62 ± 10.76	0.369
Maximum Length of the Femur (mm)	434.38 ± 21.64	455.91 ± 25.29	**<0.001**
Physiological Length of the Femur (mm)	430.13 ± 21.64	453.25 ± 25.05	**<0.001**
Anterior-posterior width (dry bone) (mm)	32.47 ± 3.45	35.84 ± 4.51	**<0.001**
Femoral Head Diameter (dry bone) (mm)	41.38 ± 2.62	45.43 ± 3.16	**<0.001**
GT-FC (dry bone) (mm)	86.88 ± 5.91	101.22 ± 34.60	**<0.001**
Medial Cortical Thickness (mm)	4.84 ± 0.29	5.38 ± 0.22	0.148
Lateral Cortical Thickness (mm)	4.62 ± 0.17	5.31 ± 0.14	**0.003**
Anterior Cortical Thickness (mm)	3.83 ± 1.06	4.04 ± 1.25	0.445
Posterior Cortical Thickness (mm)	3.23 ± 1.33	3.10 ± 1.40	0.68
External Femoral Neck Diameter (mm)	32.48 ± 2.71	36.62 ± 3.57	**<0.001**
Internal Femoral Neck Diameter (mm)	28.48 ± 2.89	32.17 ± 3.80	**<0.001**
Bending Strength (mm^4)	22.62 ± 9.26	36.28 ± 14.65	**<0.001**

Tortional Strength (mm^4)	45.25 ±18.52	72.56 ± 29.31	**<0.001**
GT-FC (scan) (mm)	91.34 ± 6.31	102.02 ± 8.56	**<0.001**
Femoral Head Diameter (scan) (mm)	40.32 ± 2.30	44.65 ± 3.66	**<0.001**
Anterior-posterior width(scan) (mm)	40.32 ± 2.30	44.65 ± 3.66	**<0.001**
Fractal Dimension of Femoral Head (ROI 1) (FD)	1.52 ± 0.10	1.50 ± 0.09	0.943
Fractal Dimension of Femoral Neck (ROI 2) (FD)	1.42 ± 0.10	1.42 ± 0.12	0.813
Fractal Dimension of Trochanteric Region (ROI 3) (FD)	1.49 ± 0.78	1.48 ± 0.10	0.098
Total Area (mm^2)	440.05 ± 52.11	541.99 ± 77.27	**<0.001**
Cortical Area (mm^2)	62.51 ± 17.47	67.77 ± 17.46	0.089
Cancellous Area (mm^2)	377.99 ± 46.83	474.22 ± 68.07	**<0.001**
Percentage Cortical to Total Area (%)	16.72 ± 5.09	14.33 ± 3.27	**0.017**
Percentage Cancellous to Total Area (%)	85.81 ± 3.39	87.53 ± 2.47	**0.017**

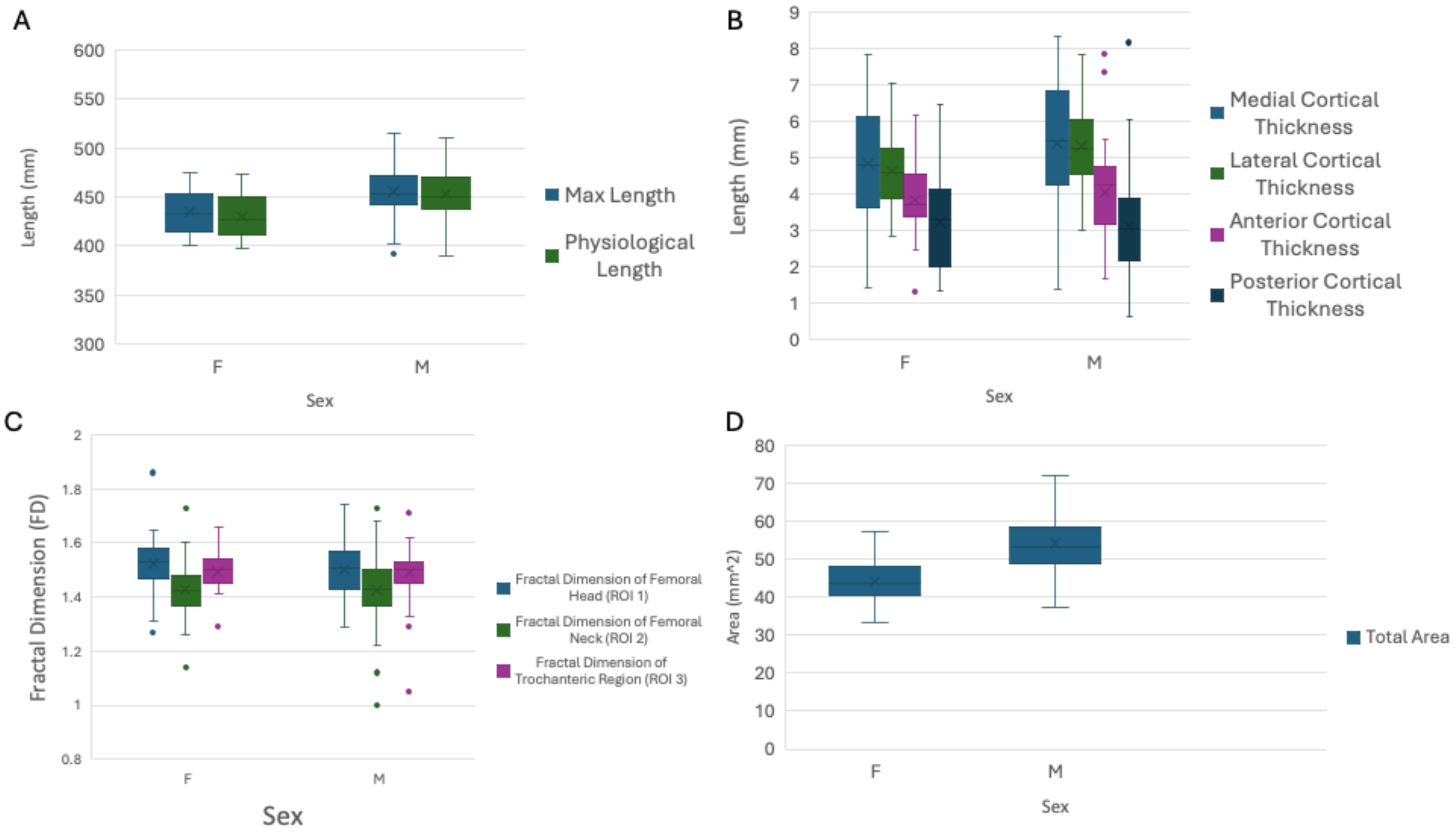

Figure 6: Measurement sizes between females and males in different parameters. Panel A: the differences in maximum length and physiological length. Panel B: the differences in medial, lateral, anterior, and posterior thickness. Panel C: the differences in fractal dimensions across the femoral head (ROI 1), femoral neck (ROI 2), and trochanteric region (ROI 3). Panel D: the differences in total Area. F is defined as female and M is defined as male.

Logistic Regression Modelling

Results of the crude binary logistic regression analysis revealed that the maximum length of the femur, physiological length of the femur, GT-FC (dry bone), external femoral neck diameter, internal femoral neck diameter, bending strength, torsional strength, GT-FC (scan), femoral head diameter, total area, and cancellous area met the criteria for possible model candidates (this included have a significant difference between the sexes and having a correct sex classification of 70%). These variables were utilised in an all-subset regression analysis to determine the best predictive model for sex estimation. The results revealed two models (Table 7): the model including one variable (cancellous area) had the best score for BIC (Model 1), while the model with three variables (cancellous area, maximum length of the femur and physiological length of the femur) scored the best according to the AIC (Model 2).

Table 7: Logistic regression equations for sex estimation

Model	Variables	Equation
1	Cancellous area (mm^2) – CA	$P(\text{Male}) = \frac{e^{(0.32*CA)-(12.61)}}{1+e^{(0.32*CA))-(12.61)}}$
2	Cancellous area (mm^2) – CA Maximum length (mm) – ML Physiological length (mm) – PL	$P(\text{Male}) = \frac{e^{(0.32*CA)-(0.31*ML)+(0.30*PL)-(7.49)}}{1+e^{(0.32*CA)-(0.31*ML)+(0.30*PL)-(7.49)}}$

These two models were further assessed to determine their suitability for sex estimation. Model 1 had a pseudo-R-squared value of 0.539 and a correct classification percentage of 82.2% overall, while the multivariate model with 3 variables had a pseudo-R-squared value of 0.583 and a correct classification percentage of 84.1% overall. Assessment of sensitivity and specificity indicated the best sectioning-point (maximising both sensitivity and specificity) for both models was 0.6 (Figure 7). The sensitivity refers to the ability of a model to correctly identify positive cases, otherwise also known as the true positive rate. The specificity refers to the ability of the model to correctly identify negative cases, otherwise also known as the true negative rate. For both models, if the probability is greater than 0.6, the individual is classified as male. When the probability is lower, the individual is classified as female. Re-running the models with this adjusted cutoff value, slightly decreased the correct classification percentage of Model 1 from 82.2% to 81.1%. However, the correct classification percentage for Model 2 increased from 84.1% to 86.4%.

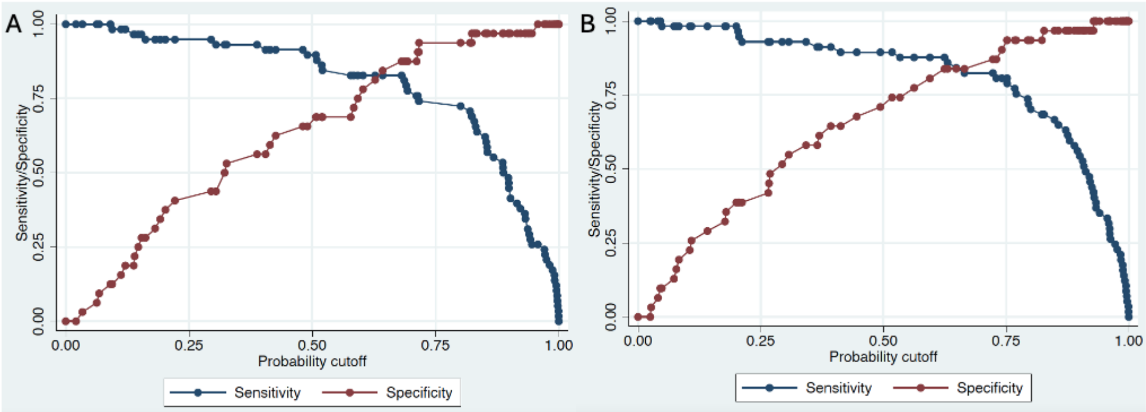

Figure 7: Sensitivity versus specificity graphs for the two models generated. Panel A: The graph for the model with one variable (cancellous area); Panel B: the graph for the model with three variables (cancellous area, maximum length of the femur, and physiological length of the femur).

Application of models on forensic cohort

The models were applied to the forensic cohort and the results are summarized in Table 8. The respective variables required for each model were measured and used in both models. Both models showed an 88.2% agreement with previous estimates (i.e., the result of the model, had the same result as the FACT estimate). Regarding model 1, where sex had previously been estimated, the model assigned the same sex in all but two individuals. The two discrepancies show two different results, where UCT 352 shows a strong probability of the individual being classified as male (as it is above the sectioning point of 0.5), whereas UCT 631 scored just below the sectioning-point of 0.6, thereby classifying the individual as female. Similarly, regarding model 2, where sex had previously been estimated, the model assigned the same sex in all but two individuals. Discrepancies arise in UCT352, where the probability was quite high classifying the individual as male (above the sectioning point of 0.6); similarly, in UCT354, the probability is strongly classifying the individual as female as it is well below the sectioning-point of 0.6. A probability for UCT401 using model 2 was not generated as all measurements could not be taken from the bone due to damage, which would not give an accurate reflection of the method applied.

Table 8: Summary of the results of the selected models on the forensic cohort.

UCT number	FACT estimate	Probability of Model 1	Outcome	Probability of Model 2	Outcome
UCT 316	Indeterminate adult	0.99	M	0.99	M
UCT 352	F	0.89	M	0.88	M
UCT 353	M	0.99	M	0.99	M
UCT 354	M	0.67	M	0.43	F
UCT 356	Indeterminate adult	0.89	M	0.93	M
UCT 362	Indeterminate adult	0.99	M	0.99	M
UCT 366	M	0.96	M	0.93	M
UCT 371	M	0.79	M	0.84	M
UCT 400	M	1.00	M	1.00	M
UCT 401	M	0.69	M	-	-
UCT 407	Indeterminate adult	0.97	M	0.97	M
UCT 416	M	0.98	M	0.99	M
UCT 442	M	0.95	M	0.95	M
UCT 452	M	0.94	M	0.78	M
UCT 564	M	0.99	M	0.98	M
UCT 573	Indeterminate adult	1.00	M	1.00	M
UCT 574	Indeterminate adult	1.00	M	1.00	M
UCT 604	M	0.98	M	0.97	M
UCT 623	M	0.70	M	0.69	M
UCT 626	M	1.00	M	0.99	M
UCT 629	Indeterminate adult	0.62	M	0.64	M
UCT 630	Indeterminate adult	0.99	M	0.99	M
UCT 631	F	0.69	M	0.59	F
UCT 633	Indeterminate adult	1.00	M	1.00	M
UCT 634	Indeterminate adult	0.93	M	0.91	M
UCT 636	Indeterminate adult	0.47	F	0.29	F
UCT 640	M	0.96	M	0.95	M
UCT 642	M	0.95	M	0.95	M
UCT 672	Indeterminate adult	0.99	M	0.98	M
UCT 674	Indeterminate adult	0.95	M	0.96	M

Legend: F = Female; M = Male; *Probability > 0.6 = M.

Chapter 4 - Discussion

Intra- and Inter-rater observations

The ICC and Cohen's Kappa statistics display levels of agreement between measurements. The results of this study ranged across all four ranges of reliability (perfect, good, moderate and weak). The variables that showed high levels of reliability included maximum length of the femur, physiological length of the femur, GT-FC (dry bone), femoral head diameter (dry bone), Anterior-posterior width(radiograph), Anterior-posterior width (dry bone), femoral head diameter (radiograph), external femoral neck diameter, as these variables had ICC values > 0.9 for both intra- and inter-rater observations. This not only speaks to the ease of collection of these variables from observer to observer but also the high reproducibility of the measurements. Both types of variables (dry bone and radiographic) are represented in this group, indicating the potential of both variable types aiding in the sex estimation workflow.

While many variables demonstrated high levels of reliability, the Singh index displayed poor agreement between measurement sets, prompting further investigation into potential contributing factors. The Cohen's Kappa values for the Singh index inter-rater observations was -0.017 (CI: -0988 -0.0649; 95% CI). This indicates that agreement is less than by random chance, as there was only one case in which both raters had the same result between measurement sets. Hauschild et al. (2009) similarly found poor results for inter-rater agreement. Possible reasons for discrepancies could be due to inadequate capture of the cancellous bone when generating the radiographs. Hauschild et al. (2009) express a similar sentiment and contribute that this is due to radiography's lower resolution compared to the screen film technique. Radiographs in this study were produced via a LODOX Statscan instrument, which has been reported to have minimal distortion and can detect skeletal trauma (Mamabolo, Alblas & Brits, 2020; Stull, L'Abbé & Steiner, 2013). A more plausible reason could be the issue of differentiating between scores in the Singh Index (Koot, Kesselaer & Clevers, 1996), as can be seen by the radiographs presented in (Singh, Nagrath & Maini, 1970).

The Cohan's Kappa value for the Singh index of intra-rater observation was 0.353 (CI: 0.1302-0.5748; 95% CI), which indicates fair agreement. Koot, Kesselaer and Clevers (1996) and Wachter et al. (2001) both found strong/adequate agreement when it came to intra-observer agreement, which is not in agreement with the result of this study. It is important to note however that Koot, Kesselaer and Clevers (1996) made use of a sample size of 10 individuals for intra-observer error compared to the total 80 individuals used in the study. Their smaller sample could skew the results of their study. The current study and Hauschild et al. (2009) found poor to moderate agreement (except for one rater which had excellent agreement). The discrepancies in results between this study and other studies could be due to the different radiography modalities used which could have affected the Singh Index scoring, as the parameters used to take the radiographs of this study could differ from the ones taken in the original study where the method was developed (they made use of traditional normal exposure x-rays) (Singh, Nagrath & Maini, 1970). Differences in the level of expertise of the radiographer could also affect the assessment of this index, resulting in discrepancies in scores (Hauschild et al., 2009; Koot, Kesselaer & Clevers, 1996).

The above-mentioned studies showed discrepancies in their results regarding the Singh Index's intended purpose (the ability to evaluate the degree of osteoporosis in individuals). The current gold standard for measuring osteoporosis is accomplished using dual-energy X-ray absorptiometry. This is due to DEXA having high accuracy rates, as well as precision errors (Kanakaris & Lasanianos, 2014). In this study, the Singh Index was utilized to observe whether there was an association between Singh index scores and the sex of the individual. The statistical analysis indicated there were no significant differences between scores for females and males. Sample size could be the reason for no association noted, as there were more males included in this study compared to females. It is also important to note the intended purpose of the Singh Index; with this in mind, future research could factor in age and observe if there is an interaction between these three variables (Singh Index scores, sex, and age).

Sexual dimorphism studies on dry bone measurements

When observing the dry bone measurements of this study (maximum length of the femur, physiological length of the femur, AP length of the proximal femur, femoral head diameter, and length of the proximal femur), males had larger measurement values on average for each variable. Significant differences between males and females were found in all dry bone measurements ($p < 0.001$). This indicates a great degree of sexual dimorphism in the femur at a macroscopic level. King, İşcan and Loth (1998), İşcan and Shihai (1995) and Asala, SA (2001) found similar results in that males had larger measurements on average overall. King, İşcan and Loth (1998) examined sex differences within a Thai population, revealing significantly larger measurement values in males across various dimensions such as maximum length and vertical head diameter.

Similarly, İşcan and Shihai (1995) investigated sexual dimorphism in a Chinese population, using six standard measurements in the femur. Their study also found that male measurement values were greater in all variables investigated, which includes the maximum length of the femur. Additionally, Purkait (2003) investigated sexual dimorphism of the femoral head, finding consistent patterns of larger measurements in males across dimensions such as maximum vertical and horizontal diameters. Macho (1990) extended this inquiry to different South African populations, underscoring not only the prevalence of larger male measurements but also advocating for population-specific approaches in sex estimation methods. Their study utilized discriminant function analyses tailored to each population, revealing the diverse expressions of sexual dimorphism. Similarly, Asala (2001) explored sexual dimorphism in femoral head dimensions within South African populations, further corroborating the trend of larger measurements in males. These findings echo the broader understanding of sexual dimorphism elucidated in previous research, reinforcing its relevance in sex estimation techniques applied by forensic anthropologists.

Comparing these dry bone measurements (AP length of the proximal femur, femoral head diameter, length of the proximal femur) to that of the same measurements on the radiographs, the results of this study also reflect that on average males had larger measurement values compared to females. Most notably, significant differences were noted between the dry bone measurements and the same measurements done on a radiograph. These differences could be due to the difference in ease of measuring a linear two-dimensional measurement, compared to a three-dimensional object with its morphological variability. Research into this discrepancy has shown that the translation of measurements from a three-dimensional object to a two-dimensional scan minimally affects the measurement (Corron et al., 2017; Robinson et al., 2008). These studies however made use of computed tomography, compared to this study. Furthermore, Corron et al. (2017) highlighted that any discrepancies could likely be due to variability in the inter-observer step, where the identification of landmarks could be different. The results of this study however prove otherwise, as great inter-observer reliability was seen for all 6 measurements (all ICC values > 0.85). Another possible reason for differences in measurements is that the landmarks used for measurement on the three-dimensional structure may not have translated well onto the two-dimensional radiographs. While discrepancies may occur due to the orientation and overall morphology of the femora, this was accounted for when setting up the femora for scanning. This was done by fixing the bones in place in the required orientation on the scanning table. Similarly, Corron et al. (2017) prompted this issue in their study and made use of a reorientation step to ensure measurements taken from scans were comparable to dry bone measurements. Their reorientation step allowed for minimal discrepancies between measurements. Another possible reason could be the distortion of certain parts of the radiographic image, which could alter measurement lengths. Stull, L'Abbé and Steiner (2013) investigated the degree of distortion of the LODOX Statscan and found minimal distortion in the y-axis. Due to the design of the LODOX Statscan, it is reported that distortion is greater in the x-axis compared to that of the y-axis. Statistical analysis of the TEM and %TEM in this study found that discrepancies between dry bone measurements and radiograph measurements were minimal, as all values were less than 1mm (all TEM values were lower than 0.6mm). The %TEM showed that distortion was greater in the x-axis, as the measurements done in the x-axis had greater %TEM (AP GT-FCand length of the proximal femur) compared to the measurements done in the y-axis (femoral head diameter).

Anthropological Studies using Radiographs

Research on anthropological analysis using radiographs has been extensively documented (Franklin, Swift & Flavel, 2016; Mamabolo, Alblas & Brits, 2020). This has included research on age estimation utilizing the femur (Lynnerup, Thomsen & Frohlich, 1990; Macchiarelli & Bondioli, 1994) and the clavicle (Walker & Lovejoy, 1985). Most of the studies have focused on the cancellous structure in correlation with age and found an inverse relationship. Sex- and site-specific cancellous bone patterns were also noted, with a more pronounced decrease in bone density seen in females. An image comparative study by de Froidmont et al. (2013) found that different image modalities fare better for different measurements. Their results highlighted better reliability of femoral head measurements using conventional X-rays while better reliability of MDCT for humeral head measurements.

Sex estimation constitutes a crucial aspect in constructing a biological profile. Given the considerable variation in sexual dimorphism among different populations, it becomes imperative to develop sex estimation models tailored to specific populations, utilizing variables capable of distinguishing between males and females. Sexual dimorphism encompasses differences in various aspects such as body size, shape, developmental pace, and behaviour within a population. The use of imaging modalities for the use of sex estimation has been well-documented and researched (Rowbotham & Blau, 2020). More specifically, radiographic imaging for sex estimation has been researched using cranial elements (Sidhu et al., 2014;

Veyre-Goulet et al., 2008) and post-cranial elements (Stull, L'Abbé & Ousley, 2017). This study investigated the use of radiographs produced via LODOX imaging and their use in estimating sex in the proximal femur. Parameters of interest were extracted from the radiographs and subjected to statistical analysis to identify whether significant differences were noted between females and males. Out of the 25 variables collected, 19 variables (76%) were collected from the radiographs and 12 of these variables (63.2%) showed significant differences between females and males.

Sexual Dimorphism in imaging-studies

Research using imaging systems to investigate sex estimation of the proximal femur have previously been conducted (Aslan et al., 2024; Ford, Kumm & Decker, 2020; Li et al., 2022). These studies generated sex estimation (both univariate and multivariate) models using a setup cohort (training data set) and later tested it on a separate cohort (test data set) (Aslan et al., 2024; Li et al., 2022). These studies highlight that models developed for specific populations generate high accuracy rates (as the rates ranged from 91% and higher). These also show that the accuracy rates for both the univariate and multivariate model generated for this study (which had accuracy rates greater than 80%) compared to the competitive rates found in the studies examined (Aslan et al., 2024; Ford, Kumm & Decker, 2020; Li et al., 2022). This, therefore, highlights the potential avenue of imaging systems in the use of anthropology.

Studies investigating changes within cancellous bone often employ advanced imaging systems such as computed tomography (CT) and dual-energy x-ray absorptiometry (DEXA). However, this study sheds light on the potential use of LODOX imaging for anthropological casework in terms of research and clinical casework. Nonetheless, studies utilizing the LODOX Statscan scanning for anthropological methodologies are novel. Walters (2016) utilized LODOX instruments to examine the presentation of certain diseases and metabolic conditions in the Kirsten Skeletal Repository at Stellenbosch University which is comprised of a large mixed ancestry population. Similarly, Lakha (2015) employed LODOX imaging to try to establish standards for epiphyseal union in South African children, obtaining radiographs from hospitals in Cape Town and Johannesburg, as well as mortuaries in Cape Town.

Additionally, Stull (2013) employed the LODOX instrument to develop sex and age estimation models for South African subadults using metric analysis based on LODOX scans. The study population comprised subadults from Forensic Pathology Services (FPS) at Salt

River and the Red Cross War Memorial Children's Hospital in Cape Town, South Africa, between 2007 and 2012. Stull, L'Abbé and Ousley (2017) explored the use of LODOX imaging and its potential use in subadult sex estimation by radiographing post-cranial elements. The current study further investigated the potential of LODOX in sex estimation, however this time with an emphasis on cancellous bone, by looking at parameters such as cancellous area, biomechanical properties of the femoral neck and fractal dimension as a proxy for cancellous volume.

The cortical thickness of the proximal femur was measured in four different areas in the present study. On average, males had thicker cortical bone at the proximal femur in the medial, lateral and anterior areas. Females however had thicker cortical bone measurements in this study in the posterior area. Despite these differences, none of them were statistically significant. Chen et al. (2010) investigated changes in cortical bone at the femoral neck in relation to age and noted a decrease in cortical thickness for both sexes, with a more pronounced loss observed in females. It is important to note that their study consisted of a total of 56 individuals and was further divided into different age categories when investigating differences, whereas this study had a total of 90 individuals and was not further divided into different age cohorts. Despite differences in regions examined, sample size and age differences between cohorts in this study and Chen et al. (2010), similarities were observed. Males on average had larger cortical thickness. Their study also highlighted decreases in cortical thickness with an increase in age. With this in mind, it could explain why the female cohort of this study had a larger posterior cortical thickness, as this cohort was younger on average (but not significantly younger) compared to the male counterpart.

Exploring the results of the biomechanical properties of the femora used in this study, the results highlighted that on average, males had a greater bending and torsional strength compared to that of the female cohort in this study. These biomechanical properties are functionally responsible for allowing the femur to withstand loads and forces applied to it. In turn, males had greater external and internal femoral neck diameters. Statistical analysis of these differences showed that differences were significant between the two sexes. Riancho et al. (2007) investigated biomechanical indices of the femoral neck using dual-energy x-ray absorptiometry (DEXA) and found similar results. Their study highlighted the femoral neck width was greater in males compared to their female counterparts. The section modulus, which was defined as the bending resistance related to the diameter and distribution of the given material, was investigated in their study and found that with increasing age in women, the section modulus/bending resistance tended to decrease. In males, it tended to remain stable.

This study examined three different aspects of the area of the proximal femur. Bone area and shape play an important functional role in the strength of bone to prevent fractures from occurring (Macdonald et al., 2011; Riggs et al., 2004). The results highlighted that on average, males had larger total, cancellous and cortical area measurements compared to the females in the cadaveric cohort. Statistical analysis of these differences indicated that total area and cancellous area were significant between males and females ($p < 0.001$). This pattern of males having larger areas is a common finding. Macdonald et al. (2011) found similar results when comparing area measurements between males and females. They found that the total area and cortical area at the distal radius and distal tibia were greater in males compared to females, with the differences between the two groups being significant ($p < 0.001$ between all age groups). Another key difference is that they investigated the difference in a set age range (20-29 years of age); whereas this study looked at a broader age range (20-65 years of age).

Riggs et al. (2004) found similar results when investigating the area in the femoral neck. Similar to Macdonald et al. (2011), they made use of QCT and compared the differences between the sexes in the age groups of 20-29 years of age. They found significant differences between the cortical bone area and the cancellous bone area at the femoral neck. Despite the different imaging modalities and age ranges used in the two studies highlighted, all three studies were able to highlight the differences in areas between the two sexes.

The phenomenon of sexual dimorphism arises from a combination of intrinsic factors like genetic influences, activity patterns and hormone levels (Gilbert et al., 2000; Hughes et al., 1996; Meunier et al., 1994; Ruff, 1987) as well as extrinsic factors including nutrition and cultural behaviours (Stinson, Bogin & O'Rourke, 2012). Numerous studies have demonstrated sexual dimorphism in different populations, particularly in the femur (Asala, SA, 2001; Chen et al., 2010; Eckstein et al., 2007; İşcan & Shihai, 1995; King, İşcan & Loth, 1998; Macho, 1990; Milner & Boldsen, 2012; Purkait, 2003; Riancho et al., 2007) underscoring its utility as a tool for sex estimation techniques employed by forensic anthropologists. This study further highlights the differences between sexes in the femur. The femur showed sexually dimorphic traits in both dry bone and radiographic measurements.

Fractal Analysis and Sexual Dimorphism

The research into the use of fractal analysis for sex estimation is novel and limited research on the topic exists. Previous research using fractal analysis (FA) in forensic sciences focused on age-at-death estimation techniques (Kucheryavski, Belyaev & Fominykh, 2009; Obert et al., 2014; Obert et al., 2017), antemortem ageing techniques (Marzi et al., 2020) and post-mortem changes (De-Giorgio et al., 2022). Fractal analysis (FA) is a quantitative method to evaluate bone volume and architecture (Hayek et al., 2020; Lopes & Betrouni, 2009; Soltani et al., 2021).

This study investigated FA at three points on the proximal femur: the femoral head (ROI 1), the femoral neck (ROI 2), and the greater trochanteric region (ROI 3). This study aimed to assess whether fractal analysis could differentiate between females and males; however, statistical analysis indicated that no significant difference was present between the two sexes in all three areas examined. This indicates that there was no difference in cancellous volume and architecture between the sexes. Fractal dimensions have been previously assessed in the mandible using plain film radiographs (dos Santos Menezes et al., 2021) and CT scans (Santos et al., 2023). Significant differences were not observed using plain film radiographs, but significant differences were observed using CT. The choice of imaging modality could account for the lack of significant differences observed in this study, as CT scans tend to have fewer artefacts than radiographs. Previous studies have noted a correlation between biomechanical properties (such as compressive strength, Young's Modulus and stress) and fractal dimension, indicating its utility in measuring bone strength (Grampp et al., 1999; Sanchez-Molina et al., 2013). These studies aided in interpreting the results of this study and informed that no differences exist in biomechanical properties between females and males. Further studies could investigate whether sexual dimorphism could exist in different cancellous sections of more sexually dimorphic bones in the skeleton, such as the pelvis or skull, using fractal analysis. Another possible avenue could be to investigate the interaction between sex and age. Furthermore, future studies could look at the correlation between cancellous bone volume and age. The results of this study highlighted that the fractal analysis of the three sites looked at were not ideal to differentiate between females and males as no significant differences were found between the two groups; thus, were not considered in the logistic regression models used.

Logistic Regression Modelling

When generating the logistic regression models in this study, parameters were selected using the percentage of correct classification (>70%) and whether the crude model (variable in isolation) was significant or not. Scan measurements were selected over dry bone measurements if both variables were found to be significant for the model. This was done as it was concluded that scan variables were reasoned to be more robust as the method of measurement allowed for landmarks to be more easily identified and measurement on a 2D image is easier compared to that of a 3D object. This resulted in 11 variables being used for an all-subset analysis.

When the variables were assessed according to AIC and BIC, two suitable models were generated. The first included only the cancellous area, while the second included three variables (cancellous area, maximum length, physiological length). The sectioning-point used is a value which is used to assign the classification of the individual. If the probability falls below the sectioning-point of 0.6, the individual is estimated to be female. If the probability is above the sectioning-point, the individual is estimated to be male. After adjusting the sectioning-points for the models (following assessment of sensitivity vs specificity plots), the classification percentage for the single variable model (model 1) was 81.1%; while the classification percentage for the model with three variables (model 2) was 86.4%. These percentage changes occurred due to the shift in sectioning-point, which affected where the probability of classification of individuals. These shifts in sectioning-points also account for the uneven group sizes in the training set, while maximizing sensitivity and specificity. When applying these models to separate cohorts (in this study known as the forensic cohort) and comparing them to previous estimates generated, both models resulted in classifications >80%. These classification percentages are similar to those of Steyn and İşcan (1998), who found percentage classifications of 80.2% to 85.7% when looking at metric cranial traits. Kranioti et al. (2009) investigated femoral metric traits using radiography and found classification percentages of 82.8% to 88.6%. The results of this study and the two presented, indicate that these metric methods yield similar results compared to widely accepted and used non-metric traits (Klales, Ousley & Vollner, 2012).

A Chilean study investigated sex estimation of the proximal femur using metric methods (Carvallo & Retamal, 2020). Univariate and multivariate sex estimation models were generated. Their results highlighted that the multivariable model had overall accuracy and sex-specific accuracies ranging from 82.9%-95.7%, while the univariate models had classification percentages ranging from 81.4% - 92.9%. Curate et al. (2016) investigated sex estimation on the proximal femur and used logistic regression models. A model was generated on a test sample and correctly predicted the sex in 92% of the cohort. When applied to the holdout sample, sex was accurately estimated in 91.7% of the cohort. These two studies highlight that the classification percentages of this study are in similar ranges compared to other studies. These results are similar to that of Steyn and İşcan (1997), where the distal breadth of the femur alone had an accuracy of 90.5%; while the combination of both the of the femur and tibia measurements had an accuracy of 91.4%.

It is important to note that many of the estimates for the forensic cohort had indeterminate outcomes and their estimates relied on primarily non-metric methodology only. Klales (2020) demonstrated that metric methods perform better in terms of validity and reliability, however, does not have the ease, affordability, and speed of morphological analysis. Specificity is better elucidated in metric estimations and can highlight minor differences that non-metric methodology fails to pick up on in long bones. When comparing this to a similar study, King, İşcan and Loth (1998) found that bicondylar breadth provided the highest separation for a single variable in males at 94.3%, while maximum head diameter yielded 97.1% accuracy in females.

Limitations and Future Research Avenues

Various limitations should be considered in the interpretation of these results. Firstly, due to the makeup of the skeletal repositories in South Africa, the ideal 1:1 ratio in terms of sex could not be achieved. This limits the variability in the sample, as the cadaveric cohort consists of older individuals who have donated their remains for research purposes. This limited statistical testing within groups (aged-matched groups for females and males) as there were more males than females, which would have resulted in skewed results. Logistic regression combats this as it is robust to slight imbalances in groups. The imbalances in groups were further accounted for by adjusting the sectioning-point for the models generated to maximise sensitivity and specificity. Future studies should make use of sample sizes with varied age ranges to validate the model. Furthermore, the forensic cohort consists of individuals who have been donated to the repository by the inspectorate of anatomy in the respective metropole. As previously stated, as these individuals are unidentified, there is no demographic information (i.e., the sex, age, stature) of the individual, which in turn hinders trying to maintain a 1:1 sex ratio. It is also important to note that the data generated in this study was generated on a South African Western Cape cohort, thus the data and equations generated in this study may not be generalisable to other cohorts. Research further into the topic should also check the validity of the models on individuals with known demographics (i.e., known sex to compare estimates of the model).

It is also important to note that the model made use of both radiographic and dry bone measurements. Although the dry bone vs radiographic measurements where the comparisons were done were not included in the model, it is important to consider distortion that might play a role. The study done by Stull, L'Abbé and Steiner (2013) highlighted that distortion is greater in the x-axis compared to the y-axis (that had minimal distortion). Furthermore, the measurements in the x-axis in this study showed a high level of distortion (ranging from 6-13%).

Despite these limitations, the study opted to use radiography using a LODOX instrument as methods employed need to be cognizant of resources available in a resource-constrained environment (such as South Africa) and the availability of imaging equipment for research and practice. Imaging equipment is not always readily available for research purposes, as they are being used for clinical means. The staggering number of indeterminate individuals is caused by a knock-on effect, as there are limited resources available to carry out more robust means of identification (e.g. DNA analysis), which results in more affordable and readily available methods to be relied on (such as morphometric and morphoscopic estimation). Further research can extend into the imaging of other long bones to observe trends and differences.

Conclusion

The use of cancellous bone measures such as the Singh index and fractal dimensions did not prove useful when differentiating between sexes; however, cancellous area (i.e. measuring of the cancellous area of the proximal femur) coupled with other traditional measurements were shown to be useful. Crude analysis of variables showing significant differences between females and males resulted in two logistic regression models being generated (a univariate and multivariate model). Both models had an overall classification rate of >80%.

When applying the models to the forensic cohort, both models showed an 87.5% agreement with previous estimates. Model 2 generated in this study generated a greater accuracy classification compared to model 1, thus ideally would be used when estimating sex. Furthermore, the parameters used to estimate sex are not difficult to collect, thus making it a useful tool when estimating sex for a biological profile. Future studies should investigate traits that are population specific, i.e., develop methods that rely on sexually dimorphic traits to allow for differentiation between females and males in specific populations being observed. This principle should also be applied to other long bones such as the tibia and humerus to evaluate the degree of sexual dimorphism that exists within the South African population.

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Appendix

Appendix A – Approval letters

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
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28 August 2023

HREC REF: 381/2023

A/Prof J Friedling
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Student: BSCJAS002@myuct.ac.za

Dear A/Prof Friedling

PROJECT TITLE: CANCELLOUS BONE USE IN THE ESTIMATION OF SEX IN A SOUTH AFRICAN CADAVERIC AND FORENSIC SAMPLE-
9MASTER'S CANDIDATE-MR JASON BOSCH)

Thank you for your response letter addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 August 2024.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Mr Jason Bosch will also be involved in this study.

Please quote HREC REF 381/2023 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of

HREC/ref 381.2023

Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/ref 381.2023

Western Cape Government
Health
FOR YOU

DIRECTORATE: Forensic Pathology Service
ENQUIRIES: Professor LJ Martin MB BCh *Wits* Dip For Med *SA*
M Med Path (Foren) *UCT* F C For Path *SA*
Email: lornaj.martin@uct.ac.za

Please follow instructions written in blue *italics* and delete these when the letter is completed (including this textbox). If this letter is being completed by a delegate of Prof Martin, please change the contact details in the header and name that appears throughout this letter to reflect the details and name of the delegate.

To whom it may concern,

I, Lorna J. Martin, **do / ~~do not~~** (*delete which is not applicable*) hereby grant final permission for the following researchers to have access as specified for the research project as stipulated:

Principal Investigator: Jacqui Friedling
Staff number/~~affiliation~~: 01415627

Researcher: Jason Bosch
Student number~~/staff number/affiliation~~: BSCJAS002

Project Title: Cancellous bone use in the estimation of sex in a South African cadaveric and forensic sample

Access to:

✓	*Please tick all that apply*
	The autopsy allocations
	The Office Autopsy Database and related records
✓	Forensic Pathology Services Laboratory, Salt River for observation and collection of data
	Forensic Pathology Services Laboratory, Salt River for the collection of tissue samples
	Forensic Pathology Services Laboratory, Salt River for conducting Interviews
	Forensic Pathology Services Laboratory, Salt River for obtaining informed consent

For the data collection period of 01/09/2023 to 31/12/2023

pp Signed by candidate

Professor Lorna J. Martin *(Signature)*
Head of Division
Division of Forensic Medicine and Toxicology

17/02/2023
Date *(dd/mm/yyyy)*

Approved
Signed by candidate

Ms V Thompson (*signature)*
Director
Forensic Pathology Service: WCGHW

2023/09/19
Date *(dd/mm/yyyy)*

University of Cape Town • iYunivesithi yaseKapa • Universiteit van Kaapstad

Division of Forensic Medicine & Toxicology
Faculty of Health Sciences, UCT
Anzio Road, Observatory, 7925
P O Box 13914, Mowbray, 7705
Tel: +27 21 406 6412
Fax: +27 21 448 1249
www.forensicmedicine.uct.ac.za

FORENSIC PATHOLOGY SERVICE

"Our Mission is to be an outstanding teaching and research university, educating for life and addressing the challenges facing our society."

Application to Study the UCT Human Skeletal Repository

Department of Human Biology, Faculty of Health Sciences

____ This application form is required accompanied by a 2-page research proposal and CV of the applicant. The application will be assessed by a committee, application turnaround time is aimed to be less than 6 weeks. Application that involve destructive sampling or imaging will require Human Ethics approval and may require permission from the appropriate Heritage agency before the sampling can take place. The proposal should include the study purpose, its aims and objectives, the skeletal sample required, the methods/techniques/procedures that will be used in the analyses of the selected sample, and potential outputs.

SECTION I: DETAILS OF APPLICANT			
Surname	Bosch	**Title**	Mr
First Name(s)	Jason		
Telephone	0813079134		
E-mail	Bscjas002@myuct.ac.za		
Degree for which you are currently registered/occupation	MPhil (Biomedical Forensic Sciences)		
Supervisor/PI	**Title & name**	Associate Professor Jacqui Friedling	
	E-mail address	jacqui.friedling@uct.ac.za	
Institution	The University of Cape Town		
Department/School	Forensic Medicine		
Student number	BSCJAS002		
Physical address		16 Myrtle Road	
		Bishop Lavis	
		7490	
		Cape Town	

SECTION II: ACADEMIC QUALIFICATIONS OF APPLICANT		
Qualifications (completed degree/s and/or degree/s in progress)		
Institution	**Degree**	**Date Awarded**

The University of Cape Town The University of Cape Town	BSc BMed (Hons)	13 July 2021 23 March 2022

Purpose of study (dissertation, publication, etc.)
Dissertation

If any additional tools are required for the proposed study (e.g. magnification kits, cameras, scales, callipers etc.), the applicant is expected to provide the necessary equipment themselves or plan with the Department of Human Biology.

SECTION III: NATURE OF STUDY	
Title of project	Cancellous bone use in the estimation of sex in a South African cadaveric and forensic sample
Date on which you intend to commence research/data collection	1st May 2023
Date on which you intend to complete research/data collection	31st August 2023
Total number of skeletons to study	130
Skeletal elements to be analysed (e.g. teeth, crania, long bones, full skeleton etc.)	Femurs + Pelvic bones
Do you intend to photograph or radiograph any of the samples? If yes, please explain the purpose/s	Yes (radiograph). To visualise the trabecular bone in the proximal femur using Lodox scanning.
Do you intend to do any invasive or destructive sampling?	No
Total number of individuals to be sampled	-
Region of skeleton to target, and amount required for sampling	-
Explain purposes for destructive/invasive sampling, as well as the methods to be followed.	-

All applicants conducting research on Khoesan remains within the repository are expected to read the San Code of Research Ethics before commencing data collection and should, additionally, be familiar with the acceptable scientific terminology/classifications for the population in question.

Read more about the San Code of Research Ethics (South African San Institute, 2017) by following the link below:

http://trust-project.eu/wp-content/uploads/2017/03/San-Code-of-RESEARCH-Ethics-Booklet-final.pdf

I have read and understood the San Code of Research Ethics ☑

SECTION IV: OUTREACH/COMMUNITY ENGAGEMENT
Please outline how this research will be fed back into the community, or how you intend on engaging with the public to make your research accessible
The expected outcome for the study is that the models and results generated from this study can assist in the generation of biological profile (sex estimation) when bones that are regularly used for sex estimation are not available. This will assist in identification of unknown individuals.

Signature of Applicant: Date:

As curator I met with the student and dicussed the situa
submitted to ethics. He was unaware his supervisor ha

Signed by candidate

January 2023

____________________ ____________________

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Is the proposed project feasible?	
Has the application met all expectations? (aims, methods, community engagement etc.)	
Comments	There is an irregularity in that the student began working in the repository before final signed approval was received. Proof of ethics was sent to the student and supervisor on 28 August, but only received by the curator on September 12th. The student was working in the repository from September 7. As curator I met with the student and discussed the situation: a study deviation will need to be submitted to ethics. He was unaware his supervisor had not submitted the Proof of ethics. In future, the signed repository approval form must be provided with any request for access to the repository.

Approved by: Victoria Gibbon

Signature: Signed by candidate Date: September 19, 2023

Copies of any publications or dissertations stemming from the research conducted on the UCT Human Skeletal Repository are to be forwarded to the Department upon completion.

Once permission is granted applicants should notify and send a reminder 2 weeks in advance of pending arrival, also detailed arrangements of access must be organised 2 days in advance with Ms. Caroline Powrie caroline.powrie@uct.ac.za Hours of operation are Monday-Friday 8:00-16:00.

Appendix B - Crude analysis results

Table B1: Results from crude binary logistic regression models.

Variable	Model Significance	Percentage Correct	Pseudo-R^2 value
Max Length of the Femur (mm)	<0.001	73.9	0.213
Physiological Length of the Femur (mm)	<0.001	73.9	0.242
AP width (dry bone) (mm)	<0.001	70	0.188
Femoral Head Diameter (dry bone) (mm)	<0.001	78.9	0.406
Length Proximal Femur (dry bone) (mm)	<0.001	83.3	0.459
Medial Cortical Thickness (mm)	0.145	64.4	0.032
Lateral Cortical Thickness (mm)	0.003	67.8	0.125
Anterior Cortical Thickness (mm)	0.437	65.9	0.01
Posterior Cortical Thickness (mm)	0.669	65.1	0.003
External Neck Diameter (mm)	<0.001	72.2	0.378
Internal Neck Diameter (mm)	<0.001	72.2	0.29
Bending Strength (mm^4)	<0.001	80	0.312
Tortional Strength (mm^4)	<0.001	80	0.312
Proximal Femur Length (scan) (mm)	<0.001	80	0.427
Femoral Head Diameter (scan) (mm)	<0.001	75.6	0.395
AP width (scan) (mm)	0.002	68.6	0.143
Fractal Analysis of Femoral Head (FD)	0.294	65.6	0.017
Fractal Analysis of Femoral Neck (FD)	0.942	64.4	0.000
Fractal Analysis of Trochanteric Region (FD)	0.866	64.4	0.000
Total Area (mm^2)	<0.001	81.1	0.487
External Area (mm^2)	0.161	64.4	0.03
Internal Area (mm^2)	<0.001	82.2	0.539
Percentage External to Total Area (%)	0.007	63.3	0.105
Percentage Internal to Total Area (%)	0.007	63.3	0.107

Appendix C – Stata output for all-subset regression models

Table C1: Stata output following all-subset regression for best regressions at each predictor quantity. Underlined and bolded values indicate the lowest value for the AIC and BIC criterion.

# Predicators	LL	AIC	BIC
1	-35.21742	74.43485	<u>**79.38952**</u>
2	-34.57659	75.15317	82.58519
3	-32.84239	<u>**73.68477**</u>	83.59412
4	-32.03586	74.07172	86.45841
5	-31.10078	74.20156	89.06558
6	-30.80565	75.6113	92.95266
7	-30.61521	77.23041	97.04911
8	-30.52867	79.05734	101.3534
9	-30.45794	80.91587	105.6892
10	-30.44178	82.88355	110.1343
11	-30.43901	84.87803	114.6061

1: Cancellous Area

2: Trabecular; Bending Strength

3: Cancellous Area; Max Length; Physiological Length

4: Cancellous Area; Total Area; Max Length; Physiological Length

5: Cancellous Area; Total Area; Max Length; Physiological Length; Bending Strength

6: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (scan); Bending Strength

7: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (Scan) Femoral head diameter (scan); Bending Strength

8: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (scan); Femoral head diameter (scan) Bending Strength; Tortional Strength

9: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (scan); Femoral head diameter (scan); Bending Strength; Tortional Strength; Internal Neck Diameter

10: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (scan); Femoral head diameter (scan); Bending Strength; Tortional Strength; Internal Neck Diameter; External Neck Diameter

11: Cancellous Area; Total Area; Max Length; Physiological Length; Proximal Femur Length (scan); Femoral head diameter (scan); Bending Strength; Tortional Strength; Internal Neck Diameter; External Neck Diameter; AP Length Lesser Trochanter