Validation of the Iscan method in clinical MSCT scans specific to an Australian population

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Declaration

I declare that the research presented in this thesis, for the Master of Forensic Science at the University of Western Australia, is my own work. The results of the work have not been submitted for assessment, in full or part, within any other tertiary institute, except where due acknowledgement has been made in the text.

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Abstract

Contemporary research in the field of forensic anthropology has been diversified and aided by the use of medical imaging technology, which is increasingly being utilised to produce estimates of chronological age as part of the biological profile of an unidentified deceased individual, or a living individual whose age is contested. Age estimates produced for living individuals are associated with significant legal and evidentiary value in cases that require a determination of criminal culpability, and need to be accurate to prevent violations of human rights. Virtual skeletal reconstructions have been empirically validated as an appropriate alternate source of biological data that may be used in lieu of traditional skeletal collections for the creation of population specific standards; the latter and have been found to increase the accuracy with which aspects of the biological profile may be estimated.

The primary aim of the present study is to develop age estimation standards for a contemporary Western Australian population as based on the analysis of virtual reconstructions of the fourth right rib using the Phase ageing method developed by Iscan et al (1984b, 1985). This involved assessing the reliability of a virtual rib visualisation protocol, and determining if age related metamorphosis of the fourth right sternal rib follows the same pattern of progression in a Western Australian population, when compared to the American population described by Iscan. Standards developed for the fourth right rib were also applied to right ribs three and five, to determine the overall applicability of the method to ribs other than the fourth.

The study sample comprises 335 clinical multi-slice computed tomography scans representing individuals (179 males and 156 females; 10 to 80 years of age) that presented at hospitals within the Perth metropolitan area. Age assessment is performed following the reconstruction and visualisation of the relevant rib using the sex specific standards provided by Iscan (1984b, 1985) (photographs and descriptions of rib morphology) in conjunction with rib reference casts (Iscan et al 1993).

The level of intra- and inter- observer accordance associated with Phase estimates are quantified prior to data collection. Intra –observer error is substantial ($K=0.76$), and inter-observer error is almost perfect ($K=0.825$). Descriptive statistics obtained for

males were comparable to those presented by Isçan for Phases 3 and higher, with more variability occurring in the female sample. A one way repeated measures ANOVA with follow up paired samples *t*-tests is performed to determine whether the mean estimated age at rib pairs (three and four, three and five, four and five) differed. Mean estimated age differs significantly between ribs three and four, and four and five within the male sample. No sex specific variation in mean estimated age at right ribs three, four and five is evident. A bivariate Pearson's product moment correlation demonstrates a strong, positive, statistically significant ($p < 0.001$) linear relationship between estimated Phase scores at rib pairs (see above), and between estimated and chronological age for each rib examined.

Linear, polynomial and multiple regression analyses are then performed to produce models for the estimation of chronological age from Phase scores for ribs three through five. The prediction accuracy of the linear models ranges ± 10.62 to ± 11.55 years for males, and ± 10.65 to ± 11.75 years for females. The accuracy of predictive linear models increases with cubic models (between ± 10.59 and ± 11.61 years for male individuals, and ± 10.45 and ± 11.47 years for female individuals). Multiple linear regressions utilising Phase scores from all three ribs produced models with the highest predictive accuracy (± 10.04 years for male and ± 9.81 years for female individuals), with comparable results achieved using enter and stepwise methods. The accuracy of these models is comparable to those based on the analysis of the dentition for a Western Australian population, and for other dental and skeletal methods in different global populations.

Transition analysis is used to evaluate the mean age at transition between Isçan age Phases to generate standards that describe the rate of morphological change in the sternal rib in a Western Australian population. The mean age for initial rib metamorphosis (transition between Phases 0 and 1) varies across samples and rib specimens. Standard deviation scores across ribs and samples indicates widespread variability in the mean age at transition between Phases that begins at the transition between Phases 2 and 3. Further research is required to quantify these variations.

This study provides a novel set age of skeletal reference standards for the estimation of age from the fourth right rib, and further validates the utility of MSCT based techniques as a credible method of analysis in forensic anthropology. These standards are directly applicable to a Western Australian population, with potential to be used to estimate age within the broader Australian populace.

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I am forever grateful to my husband Szymon Blaszkowski for being an unmoveable bulwark in the face of stress induced breakdowns, fits of self-doubt and sheer exhaustion. Thank you for your endless encouragement in everything I do.

Dedication

I would like to dedicate this thesis to my parents, Iwona and Edward Kopczyk. I can say with complete certainty that I would not be where I am today without your bravery, self-sacrifice and dedication to improving my quality of life. I hope that this thesis comes to represent all that you have wished to provide for me.

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Chapter One:

Introduction to the current study

1.1 Introduction

The discipline of forensic anthropology is best conceptualised as the application of principles of physical anthropology to a medico-legal context, for the purposes of personal identification and interpretation of circumstances surrounding death for a given set of human skeletal remains (Cattaneo 2007, Ubelaker 2008; 2010). The role of the contemporary forensic anthropologist is diverse and includes evidence recovery, species identification (differentiation between human and non-human remains), determination of the post-mortem interval and the effect of taphonomic agents on bone preservation, evaluation and reconstruction of skeletal trauma, and the construction of a biological profile (Cattaneo 2007; Scheuer 2002; Ubelaker 2008). The contribution and expertise of a forensic anthropologist is especially relevant to investigations involving human rights violations and mass disasters (natural or otherwise), where ante-mortem data is lacking, or severe fragmentation, burning, or commingling of remains may have occurred (Blau & Briggs 2011; Cattaneo 2007).

1.2 The biological profile

The biological profile facilitates the process of identification through the assessment of attributes such as sex, age, stature and ancestry (the geographical provenance of individuals), that are traditionally estimated through the direct observation and analysis of skeletal morphology indicative of those attributes (Brace 1995; Cattaneo 2007; Scheuer 2002). Other unique characteristics, such as stress markers, skeletal pathology or trauma can also contribute to the final composition of the biological profile (Cattaneo 2007).

Ultimately, the biological profile serves to reduce the pool of potentially matching identities in the case of a missing or unidentified individual. Standards derived from documented skeletal collections (individuals of known sex, age and ancestry at death) are generally used to compile the biological profile (Komar and Grivas 2008). The accuracy of a given biological profile depends on a number of factors, including what skeletal elements are available for analysis, the state of preservation of those elements (Scheuer 2002), the nature of the death under investigation (Franklin 2010), choice of

methodology, statistical analysis procedure (single versus multifactorial) and use of population specific reference standards (Ubelaker 2008; White et al 2012) (see below and Chapter Two).

1.2.1 Age as a tenet of the biological profile

The goal of age estimation is to provide the most accurate estimate of the chronological age of the individual at death based on their biological age (Ubelaker 2008). Biological age is defined as the physiological condition of the individual reflected in the state of their skeletal remains, which is correlated with chronological age (represented by the calendric time since birth) (Garvin et al 2012). The strength of this correlation is mitigated by a number of environmental and genetic factors, which generally cause the association between biological and chronological age to decrease with advancing age, thus resulting in broader age estimates (Cunha et al 2009; Garvin et al 2012). Individuals within a population may exhibit different biological ages at a single chronological age due to individualistic factors that influence senescence, such as physical activity, general health and nutrition (Garvin et al 2012). As such, forensic age estimates represent the biological (skeletal) age of a given individual, from which chronological age is inferred.

In general, the process of age estimation involves examination of morphological features present in the remains, comparison with data generated from recent documented populations, and estimation of the inaccuracy likely to be associated with a given analysis (White et al, 2012). A reliable skeletal indicator of age is defined as one that displays progressive age related change that can readily be classified based on the appearance of degenerating characteristics (in adult individuals), and can be identified as undergoing consistent morphological change in the majority of individuals belonging to a given group (Milner and Boldsen 2012). The application of a particular method differs based on whether the remains in question are judged to be juvenile (individual who has not completed skeletal growth relative to full maturity) or adult.

1.2.2 Methods for age estimation in juvenile individuals

Juvenile age estimation methods are overall considered to be more accurate than their adult counterparts because they are grounded in stable, predictable and well documented patterns of dental and skeletal growth and equilibrium (Cunha et al 2009; Scheuer 2002). However, variations in growth rates, both within and between individuals of similar and dissimilar populations are known to occur and influence the perception of biological versus chronological age (Scheuer and Black 2000). Other factors, such as chronic disease, malnutrition or nutrient deficiencies, are also known to affect the growth rate, and thus the biological age, of an individual (Franklin 2010). Juvenile dental age estimates are based on the examination of patterns of tooth formation, eruption and mineralisation, and are obtained according to the degree of completion of roots and crowns and the position of each tooth relative to the alveolar margin (Scheuer 2002, White et al 2012). Skeletal age estimates are made based on the degree of development of ossification centres and the length and size of long bones in juvenile individuals (Scheuer 2002). The use of dental and skeletal age estimation methods is complementary, given that the initial stages of epiphyseal union of some skeletal elements coincides with the completion of tooth eruption (White et al 2012).

i) Dental methods

The development of the juvenile dentition is regulated more significantly by genetic rather than environmental factors, meaning that dental age estimates are generally considered to be more representative of the chronological age of a juvenile individual (Scheuer 2002; Smith 1991). Dental aging is thus commonly used, given that patterns of tooth formation and eruption are relatively regular and predictable, and that teeth are frequently recovered in archaeological and forensic contexts in which other skeletal elements may be unavailable for analysis (White et al 2012). Teeth begin developing *in utero* at approximately 14 to 16 weeks of gestation and generally finish developing at about 18 years of age with the eruption of the permanent third molar (Hillson 1996; Mincer et al 1993); this provides a comprehensive means of estimating age prior to adulthood. However, sex and population specific variation needs to be taken into consideration when performing dental age estimates, given potential

differences in the timing of progression through stages of dental development for deciduous and permanent teeth (Hillson 1996; Moorrees et al 1963a, 1963b).

Dental age estimates are performed using a variety of techniques, including radiographic assessment of patterns of root development and mineralisation in the deciduous and permanent teeth (Moorrees et al 1963a, 1963b), macroscopic observation of tooth eruption (Franklin 2010) and comparison with visual atlases that document the development of the entire dentition (Ubelaker 1999). The Moorrees et al (1963a, 1963b) classification standards are commonly used to estimate dental age in juveniles, and involve comparison of macroscopic and radiographic data of tooth development stages to composite images for 10 permanent teeth in male and female individuals. Each developmental stage is associated with a mean age and a standard deviation of ± 2 years. Similarly, the Ubelaker (1999) standard is based on the macroscopic and/or radiographic assessment of tooth development and eruption, and is associated with a standard error of between ± 2 months and 3 years.

ii) Skeletal methods

Juvenile skeletal age estimation methods are based on the identification of skeletal elements in one of three stages of development. These include: the initial appearance of the primary ossification centre; the morphological appearance, size and shape of this centre; and the timing of fusion between primary and secondary centres of ossification (Scheuer & Black 2000). Primary ossification centres represent the initial site of ossification in the connective tissue precursor to bone during embryonic and foetal life, and generally form independently to secondary ossification centres as the diaphysis or shaft of long bones (Scheuer & Black 2000). Secondary ossification centres form at each end of the diaphysis (epiphyses) following birth, and are separated from the diaphysis by a cartilaginous growth plate (Scheuer & Black 2000). Cell proliferation within the growth plate causes the diaphysis to lengthen and expand towards the epiphyses, where cartilaginous tissue is gradually ossified (Scheuer & Black 2000); fusion between primary and secondary centres of ossification occurs when the deposition of osseous tissue exceeds the proliferation of cartilage within the growth plate (Scheuer & Black 2000). This process (known as epiphyseal fusion), occurs in a progressive and orderly manner at a known age for a variety of skeletal elements in

different populations, and can thus be utilised to estimate age in juvenile individuals based on the degree of fusion (relative to complete maturity) for a given individual (Franklin 2010; White et al 2012).

The degree of fusion between primary and secondary centres of ossification is generally quantified by scoring of the elements as unfused (non-union), unified, or completely fused (complete union) (White et al 2012). Multiple reference standards exist for a variety of postcranial skeletal elements, including the medial clavicle (Schmeling et al 2004) and bones of the hand-wrist (Greulich & Pyle 1959). These standards are associated with accuracy rates between ± 1.4 to 2.6 years, and ± 6 months, respectively. The timing of postcranial epiphyseal fusion differs according to sex, ancestry, and individualistic lifestyle factors (White et al 2012), so it is important to utilise population specific fusion standards in order to increase the accuracy of the devised age estimate. The length of long bones can also be used to estimate age, particularly when the dentition, or evidence of epiphyseal ossification, is not available (Scheuer 2002; White et al 2012). This method is not as exact compared to other skeletal and dental age estimation techniques and relies on the existence of a documented skeletal collection of similar ancestral affinity from which the unknown individual derives for comparison purposes (White et al 2012).

1.2.3 Methods for age estimation in adult individuals

Age estimation in adults is based on the analysis of patterns of skeletal degeneration and senescence (Franklin 2010; Scheuer 2002). Estimating age in adults is inherently more difficult and involves greater error as the aging process is influenced by a number of factors, such as ancestral affinity, physical activity, health status and other genetic factors (Garvin et al 2012). As such, an individual's biological age can vary across multiple skeletal elements within and between individuals (Franklin 2010). However, the application of sex and population specific standards is important to ensuring the accuracy of a given estimate. Traditional methods of age estimation in adults involve non-invasive analysis by direct observation (morphoscopic), although more destructive methods may be required in some cases (Franklin 2010). Historically popular regions for the estimation of age in adults include the pubic symphysis (Brooks & Suchey 1990; McKern & Stewart 1957; Todd 1920), auricular surface (Buckberry &

Chamberlain 2002; Lovejoy et al 1985b), and sternal rib (Isçan et al 1984b). Accuracy rates for those methods range between ± 1.53 and 12.73 years for the auricular surface (Buckberry and Chamberlain 2002), ± 2.1 and 12.2 years for the pubic symphysis (Brooks & Suchey 1990) and ± 0.5 and 10.27 years for the sternal rib (Isçan 1984a).

1.3 Sternal rib and the Isçan Phase method

The method of forensic age estimation devised by Isçan (1984b, 1985) is based on the visual (morphoscopic) evaluation of features of the costochondral junction of the fourth right sternal rib that undergoes morphological change as a function of age. The fourth rib articulates with the sternum directly via the costal cartilage and is functionally associated with facilitating movement of the rib cage during respiration and transference of lateral body weight to the axial skeleton (Scheuer & Black 2000). The utility of the sternal rib as an age marker was originally established using radiographic observation studies that identified a general pattern of osteogenesis and ossification within costal cartilages that correlated with advancing age (McCormick & Stewart 1988; Stewart & McCormick 1984).

An estimate of age using the Isçan method is made according to the shape of the rim, the appearance of alterations in the rib wall, the formation and depth of a pit in the medial articular surface of the rib, and the general density and texture of the bone (Isçan et al 1984b). An unidentified individual is assigned to one of nine Phases according to the appearance of these features and direct comparison to associated type casts, or reference photographs that depict the morphological features of each Phase. The sternal rib of a young individual (under the age of 16) is described as generally being firm and very solid, with a flat articular surface, rounded edges and a regular rim (Isçan et al 1984b). Successive changes associated with advancing age include gradual deepening of the pit from a V to a U shape, scalloping of rim edges, thinning of rib walls, an increase in the porosity of bone and the formation of sharp, bony projections and 'windows' within the walls (Isçan et al 1984b). The original Isçan et al standard is based on the analysis of documented Caucasian American males at autopsy, although separate standards have been developed using other populations and skeletal reference collections to account for individual divergences as a function of sexual dimorphism (Isçan et al 1985) and ancestral variation, specifically in the pattern

and rate of metamorphosis of discriminatory features (Isçan et al 1987; Oettle & Steyn 2000). Perceived deficiencies and limitations of the Isçan Phase method are discussed in Chapter Three.

1.4 Age estimation, skeletal collections and ‘Virtual anthropology’

Despite their historical utility in the formulation of reference standards, the use of skeletal collections poses significant challenges to the contemporary process of age estimation in adults. Skeletal collections generally comprise historic individuals that are not necessarily representative of contemporary populations due to temporal and geographical variations that differentially affect skeletal growth and degeneration (Stinson 2000). Further, the rate and degree of age related metamorphosis in skeletal elements (senescence) has been observed to vary according to individualistic cultural and socioeconomic factors that are associated with population affinity (Schmitt et al 2002). Previous studies have established that the application of out-dated or foreign standards to contemporary and/or geographically disparate populations (respectively) result in decreased accuracy in the estimation of the biological profile (Dirkmaat et al 2008; Franklin et al 2013a; Kimmerle et al 2008), thus emphasising the requirement for contemporary population specific standards.

A paucity in the availability of modern skeletal collections from which population specific standards can be formulated has acted as the impetus for the increased use of alternative forms of biological data for the development of more representative contemporary standards. Medical imaging modalities such as computed tomography (CT) are known to be an appropriate alternative source of biological data and a suitable analogue for physical skeletal collections. Radiographic methods have been utilised as a means of validating existing, and testing novel, methods for the determination of the biological profile (Franklin et al 2016), through the transposition of traditional methods to a virtual set of remains. This practice, defined as virtual anthropology (Dedouit et al 2015), utilises CT information to reconstruct a three dimensional virtual set of remains and affords several advantages to traditional approaches of skeletal analysis, including: being non- invasive and non-destructive; time efficient; and a more acceptable means of post-mortem analysis to certain religious and cultural groups (Brough et al 2012; Franklin et al 2016). A concordance

between traditional and virtual means of osteometric analysis, and thus the utility of virtual methods as a supplemental source of data, has been demonstrated (Franklin et al 2013a). Importantly, virtual methods can be used to estimate age in deceased and living individuals alike.

1.4.1 Estimating the age of living individuals

The practice of age estimation in living individuals is relatively novel in the field of forensic anthropology, although it is becoming increasingly important due to increased global migration and the resulting number of foreign individuals that cannot reliably verify their age in cases involving asylum, criminal, civil or old age pension proceedings (Schmeling et al 2007, 2008). Age estimates in living individuals are made in order to establish the probability that a given individual has attained a legally significant age threshold (Schmeling et al 2007). Traditional methods are transposed to a virtual platform, a process that is facilitated by the predominant use of CT and radiographic methods to examine the extent of skeletal or dental maturity relative to complete maturity (Hackman et al 2010; Bassed 2012). Novel methodologies for the estimation of age using a virtual set of remains include the pubic symphysis (Telmon et al 2005), auricular surface (Barrier et al 2009), and sternal ribs (Moskovitch et al 2010).

1.5 Aims and objectives

The present study is primarily concerned with the transposition of traditional morphoscopic methods of skeletal analysis to a virtual platform for the purpose of estimating age. Specifically, the study will examine the applicability and accuracy of the Isçan et al (1984b, 1985) sternal rib method in a sample of clinical multi-slice computed tomographic (MSCT) thoracic scans. MSCT scans are used as an analogue for a reference physical skeletal collection comprising individuals from a Western Australian population, given that such a collection does not currently exist. Studies utilising clinical MSCT to generate anthropological data have been performed using other anatomical sites indicative of age (e.g. Franklin & Flavel 2015; Moskovitch et al 2010), although the analysis of the morphological characteristics of the sternal rib using the Isçan method is a novel undertaking. A set of reference standards specific to a contemporary Western Australian population will be established using the data from this study, increasing the accuracy with which the Isçan method can be applied. This

research is thus forensically relevant in a Western Australian population, as it will provide novel standards for the estimation of age in unidentified living and deceased individuals. The specific aims of this project are as follows:

i) Determine whether the sternal rib can be reliably assessed in clinical MSCT scans

Statistical quantification of the error associated with data collection is a preliminary requirement prior to the commencement of the main study. Quantification of intra- and inter-observer error is especially relevant in the present study given perceived disadvantages of applying morphoscopic analytical techniques, the application of a relatively novel methodology, and varying amounts of error reported in association with the original Isçan method (1984b, 1985). The main limitation of this method is related to the nature of data obtained. Morphoscopic methods result in qualitative data that are based on the visual assessment and scoring of features, or the quantification of the presence, absence and degree of expression of a particular trait (Hefner 2009). Qualitative data are generally subjective, meaning that it is inherently associated with higher inter- and intra- observer error, is highly dependent on the experience and training of the observer, and it has not been subject to rigorous statistical analysis from which quantifiable conclusions may be drawn (Hefner 2002; Hefner & Stephen 2012).

The present study represents one of the first attempts to estimate age using the Isçan method in a sample of virtual sternal ribs derived from living individuals; quantification of observer error to establish the accuracy and legitimacy of the method is therefore required. This is especially important as the accuracy of the original method devised by Isçan et al (1984b, 1985) remains contested on the basis of perceived Phase assignment bias, a lack of quantification of intra-observer error, and a varied degree of applicability to other populations (Russell et al 1993; Matrilie et al 2007). Finally, the statistical quantification and reporting of error is recommended as best practice by the Scientific Working Group for Forensic Anthropology (SWGANTH 2013), in addition to being a requirement for the admission of expert testimony under guidelines established for forensic anthropology and other forensic disciplines (Grivas and Komar 2008).

ii) Determine if age related metamorphosis of the fourth right sternal rib follows the same pattern of progression in a Western Australian population compared to the American Caucasian population described by Isçan

Previous research has established that the use of foreign standards decreases the accuracy with which certain biological attributes can be estimated in geographically or temporally disparate populations (Franklin et al 2013b; Kimmerle et al 2008). The applicability of the Isçan standard (1984b, 1985) has been tested in several global populations (e.g. Oettle & Steyn 2000; Salem et al 2014; Yavuz et al 1998). In general, those studies demonstrate varied applicability of the original method and a tendency for it to under-estimate the age of younger individuals, and *vice versa* for older individuals in instances where consensus between actual and estimated age was reached (Oettle & Steyn 2000; Salem et al 2014). Differences in the overall appearance of the sternal rib and the pattern of morphological change as a function of advanced age have also been noted between populations (Oettle & Steyn 2000). Given the level of variation in the accuracy of the method in estimating age, and in describing the overall pattern of sternal rib morphological change in geographically disparate populations, the primary aim of this study is to determine whether the pattern of morphological change of the right fourth sternal rib in a Western Australian population follows that established in the original method (Isçan et al 1984b, 1985) and to thus concurrently evaluate its overall accuracy and applicability.

iii) Develop Western Australian reference standards for the estimation of age from the sternal rib ends as visualised in MSCT scans

There are presently no standards for the estimation of age using sternal rib Phase analysis specific to a contemporary Western Australian population. This is problematic within a forensic context as the SWGANTH recommends that standards used to estimate age should reflect the ancestral origin of the remains in question. The present study will further build upon previous research (e.g. Isçan et al 1984b, 1985) to determine the applicability of the method to other ribs (e.g. ribs three and five as opposed to exclusive use of the right fourth rib). This will increase the scope and forensic utility of the data available for the study population. As such, the final aim of this study is to develop a set of reference standards specific to a Western Australian population in order to increase the accuracy with which age estimates are performed.

1.6 Sources of data

A total of 335 MSCT clinical thoracic scans (179 male and 156 female individuals between 10 and 80 years of age) obtained from the Western Australian Department of Health Picture Archive Communication System (PACS) database will be analysed in this study. Those scans represent individuals presenting with various conditions at all public hospitals within the Perth metropolitan area. Scans are anonymised prior to receipt by the author to preserve patient confidentiality. Information regarding the sex and age of each individual is obtained from generalised patient data that accompanied each scan. Scans exhibiting ribs with complete costal cartilage ossification (adjoining the sternum), imaging artefacts or trauma were excluded from the study. Ethics approval for this project was granted on the 2 May 2016 by the UWA Human Research Ethics Office (RA/4/1/4362; see Appendix 1).

1.7 Thesis outline

Chapter Two provides an overview of methods for the estimation of age in living and deceased adult individuals, with a specific focus on the gross anatomy and embryological origin of relevant thoracic structures. This chapter is also devoted to reviewing the origins, practice and applications of virtual anthropology for the purposes of estimating age, and the methodological issues that are associated with skeletal age estimation. Chapter Three represents an analytical review of literature specifically pertinent to the development and evaluation of the sternal rib Phase aging method, and the gradual transposition of this method to a virtual platform. The materials and methods utilised in this study are presented in Chapter Four, and Chapter Five contains the relevant statistical analyses and results. Finally, a critical interpretation and discussion of the results is presented in Chapter Six, along with associated limitations and directions for future research in the field of virtual anthropology.

1.8 Limitations

The use of the PACS repository precludes the examination of the effect of ancestry (individualistic cultural factors are known to influence the process and expression of senescence) as this information is not recorded at the time that scans are acquired. Further, the use of imaging modalities (such as MSCT) are associated with a sample

bias towards individuals who present with a given condition that requires hospitalisation and treatment, or those who exhibit an underlying pathology. Those scans are therefore potentially less representative of the population from which the individual derives. The use of living individuals inherently prevents the determination of inter-method accuracy in this study (results from MSCT analysis cannot be compared to those that can be achieved using dry bone specimens from the same individual). This issue is remedied in part by the imaging study conducted by Dedouit et al (2008; see Chapter Three), although no holistic solution to this problem has yet been offered and potentially a solution may not be possible.

The analysis of virtual skeletal reconstructions also inhibits the application of tactile analytical procedures that have been identified as important in the classification of a given rib to an age Phase, where relative bone weight and its texture are important discriminant features between young and old ribs (Hartnett 2010). The importance of palpation of physical bone specimens is underscored by the analysis of the Spitalfields Cemetery population by Loth (1995), where contradictions between the appearance and 'feel' of bone resulted in a specimen being judged as older or younger based on bone weight alone. Finally, the accuracy of an analysis is (in part) determined by the quality, spatial resolution and slice thickness of the available MDCT reconstructions, where thinner slices and a higher spatial resolution favour enhanced visualisation.

1.9 Concluding summary

The current project ultimately aims to add to the existing body of knowledge concerning the use of traditional anthropological methods of analysis within a digital platform. The unavailability of a skeletal reference collection for a Western Australian population necessitates the use of clinical MSCT scans taken from living individuals to develop these standards, which will be forensically relevant for the estimation of age within this population specifically, and potentially the greater Australian population generally. Testing of the applicability of the Phase aging method over an extended age range will enhance the availability of data for estimating age in both living and deceased individuals and improve anthropological practice and forensic capabilities in Australia.

Chapter Two:

Forensic age estimation using the fourth sternal rib end

2.1. Introduction

This chapter establishes the conceptual basis for the analyses subsequently performed as part of the present thesis. The anatomical and physiological framework that underpins mechanisms of thoracic growth and senescence are considered, with particular emphasis on the embryological origin, development and function of the thorax, sternal ribs and their associated costal cartilages. This is followed by an examination of the methodological aspects of the İşcan Phase aging method, and the transposition of traditional methods of analysis to a virtual platform. Finally, methodological considerations that inform both forensic and anthropological practice are reviewed.

2.2. The anatomy of the human thorax

The human thorax is a cage like structure comprising osseous and cartilaginous tissue that is functionally responsible for the protection of the internal viscera associated with circulation, respiration, and facilitation of movements related to respiration (Scheuer & Black 2000, 2004; White et al 2012). The adult thoracic skeleton contains an anterior midline sternum, 12 pairs of laterally oriented ribs, and 12 corresponding posterior thoracic vertebrae (Scheuer & Black 2000, 2004; White et al 2012) (see Figure 2.1). Ribs 1 through 7 are referred to as ‘true’ ribs as they articulate directly with the sternum via a band of cartilage, while ribs 8 through 10 attach indirectly via the costal margin (interconnected common cartilage), and are hence known as ‘false’ ribs. The remaining ribs are referred to as ‘floating ribs’ as they are situated independently within the body wall (White et al 2012).

A typical adult rib is defined by the presence of a head, neck, tubercle and shaft (Sinnatamby 2010) (see Figures 2.2 and 2.3). The head of each rib (with the exception of ribs 1 and 10 to 12) contains two articular facets, which form synovial costovertebral joints with the body of their associated vertebrae and the vertebrae above (Sinnatamby 2010; White et al 2012) (see Figure 2.4). The neck of each rib

separates the head and the tubercle, and is crested to provide a point of attachment for the superior costo-transverse ligament (Scheuer & Black 2000; Sinnatamby 2010).

Figure 2.1. The articulated adult thoracic skeleton with associated costal cartilages. Taken from Scheuer & Black (2000).

The tubercle is located on the postero-inferior surface of each rib and presents with a medial articular facet and a lateral non articular facet; the former articulates with the transverse process of the accompanying thoracic vertebrae, and the latter provides a surface for the attachment of the lateral costo-transverse ligament (Sinnatamby 2010; White et al 2012). The shaft constitutes the curved and tapered segment of each rib between the tubercle and the sternal rib end (White et al 2012). The blunted upper border of the shaft acts as an attachment point for the internal and external intercostal muscles (Sinnatamby 2010). The medial side of the inferior edge of the shaft contains a costal groove, which in life accommodates neurovascular bundles (White et al 2012). The sternal rib represents the most anterior end of the shaft and is the point of attachment between osseous tissue and cartilage (White et al 2012).

Figure 2.2. Features of a typical rib (eighth right rib is pictured). Superior aspect pictured on the left, and inferior aspect on the right. Postero-inferior aspect is pictured in the middle. Taken from White et al (2012).

Figure 2.3. Anatomy of a typical right adult rib. Taken from Scheuer & Black (2000).

Figure 2.4. Posterior (body and transverse process of thoracic vertebra) and anterior (sternum via the costal cartilage) articulation of a typical adult right rib. Taken from Scheuer and Black (2000).

Ribs 1, 2, 10, 11 and 12 are referred to atypical ribs as they differ morphologically from the remaining ribs (see Figure 2.5). The first rib is supero-inferiorly flattened, broad, short and tightly curved in appearance, with a small head that bears only a single facet and no true costal groove within the shaft (White et al 2012). The superior surface is defined by two shallow grooves formed by the passage of the subclavian artery and the medial portion of the inferior branch of the brachial plexus (Sinnatamby 2000; White et al 2012). The second rib is morphologically intermediary between the first rib and typical ribs 3 through 9, and is distinguishable by a large tuberosity that serves as the attachment point for the serratus anterior muscle (White et al 2012). The tenth rib differs from rib 3 through 9 only in that the head usually contains a single articular facet (White et al 2012). Ribs 11 and 12 are comparatively shorter, contain a single facet on the head of the rib, and lack prominent tubercles and a costal groove (White et al 2012).

Figure 2.5. Examples of atypical adult ribs (ribs 1 and 2, and 10 to 12) and their anatomical features. Taken from Scheuer & Black (2000).

2.2.1. The costal cartilages

The costal cartilages are unossified bars of hyaline cartilage that extend anteriorly from the sternal end of ribs 1 through 10, and attach either directly or indirectly with the sternum (Scheuer & Black 2000; White et al 2012). Ribs 11 and 12 possess short, free cartilaginous ends given that they are independently positioned within the body wall (Sinnatamby 2010). The region where bone tissue merges with cartilage at the sternal rib end is referred to as the costochondral junction, and represents a developmental deviation as ossification of the cartilaginous tissue precursor to bone ceases at this landmark (Scheuer & Black 2000). Functionally, the costal cartilages serve to facilitate elasticity and mobility in the thorax, restoring the shape of the structure following expiration (Bradbury 2014; Scheuer & Black 2000, 2004). Calcification of the costal cartilages resumes at the costochondral junction with advancing age and progresses ventrally (Scheuer & Black 2000). The process of costal cartilage calcification is age and sex specific, and of potential forensic utility in the identification of unknown individuals (see below).

2.3. The embryological origin of thoracic structures

The adult ribs are derived from the sclerotome portion of somites that form within the paraxial mesoderm in the developing embryo (Cochard 2012). Somites are segmented structures that contain progenitor cells that form the osseous elements of the axial skeleton, meninges of the spinal cord, and muscles and dermis of the thoracic trunk (Schoenwolf et al 2015). Cells located in the lateral portion of sclerotome segments form the transverse processes of thoracic vertebrae and their associated ribs (Schoenwolf et al 2015). Bone develops from the condensation of primordial sclerotome mesenchyme tissue, or embryonic connective tissue that gives rise to all other connective tissues of the body (Scheuer & Black 2000). Cells within these initial condensations differentiate into chondroblasts (cartilage forming cells), that convert mesenchyme into the cartilage model precursor to bone (Cochard 2012; Schoenwolf et al 2015). The gradual replacement of this cartilage model by osseous tissue is referred to as endochondral ossification (see below) (Cochard 2012; Schoenwolf et al 2015). The overall formation of the vertebral column involves re-segmentation of the sclerotomes of somites into cranial and caudal segments, allowing the axons of motor neurons and dorsal root ganglia to rest between vertebrae (Schoenwolf et al 2015). The subsequent formation of primary ossification centres within the body, neural arch and costal processes of each vertebra facilitates the osseous development of the vertebral column (Cochard 2012). The development of vertebral arches in trunk and neck vertebrae is associated with the growth of lateral condensations of mesenchyme tissue known as costal processes (Schoenwolf et al 2015). In the thoracic region, the distal ends of these processes elongate to form ribs at approximately 5 weeks of gestation, and develop as cartilaginous precursors to bone (Schoenwolf et al 2015).

Primary ossification centres (see below) appear in ribs 5 through 7 during weeks 8 and 9 of gestation and continue to develop in a bidirectional manner (Scheuer and Black 2000, 2004). Each rib (with the exception of the 12th rib) contains a single primary ossification centre by the twelfth week of gestation (Scheuer & Black 2000, 2004). The ossification of cartilage follows the development of the primary ossification centre and progresses rapidly until the fourth month of gestation, when the foetus attains an equilibrium between the costal cartilage and bone that mirrors adult morphology (Scheuer & Black 2000, 2004). All sternal ribs (with the exception of ribs eleven and

twelve) develop from a total of four ossification centres that are present at birth (Scheuer & Black 2000). Secondary centres of ossification appear within the region of the tubercle during early adolescence (between 12 and 14 years of age) and complete fusion with primary ossification centres by 18 years of age (Scheuer & Black 2000). Finally, the epiphyses of the rib heads develop and fuse between 17 and 25 years, at which point the ribs are thereafter considered to have attained adult status (Scheuer & Black 2000, 2004) (see Figure 2.6 for a summary).

Figure 2.6. Appearance (as indicated by A) and fusion times (as indicated by F) of rib ossification centres. Taken from Scheuer & Black (2004).

2.3.1. The process of endochondral ossification

Endochondral ossification is defined as the replacement of a cartilaginous tissue template precursor to bone by osseous tissue, and represents the mode of ossification for the majority of skeletal components of the axial and appendicular skeleton (Cochard 2012; Scheuer & Black 2000; Schoenwolf et al 2015). The process requires modification of the cartilage model to promote vascular invasion, replacement of cartilage by osteoid tissue, and calcification of osteoid to gradually form bone (Bradbury 2014; Ovalle & Nahirney 2013). It is important to stress that cartilage is not converted or modelled into bone, but is rather progressively destroyed, thus providing a temporary framework for the development of more permanent skeletal structures during growth (Bradbury 2014).

Adult bone is derived from mesenchymal tissue condensations in the developing embryo, that gradually develop to form hyaline cartilage. This cartilage precursor to bone is covered by a fibrous connective tissue layer known as the perichondrium, which facilitates the growth of the cartilage model through the production of chondrocytes (Saladin 2015) (see Figure 2.7). The first step of endochondral ossification involves the direct deposition of bone by the perichondrium around the mid shaft of the bone (periosteum) (Ovalle & Nahirney 2013; Scheuer & Black 2000). The development of the periosteal collar is facilitated by a cease in the production of chondrocytes at the perichondrium and an increase the production of osteoblasts (Saladin 2015). Chondrocytes deep to the periosteum hypertrophy and die, coalescing to form a single cavity; the cartilaginous matrix simultaneously calcifies due to the presence of matrix vesicles containing calcium and phosphate ions (Bradbury 2014; Saladin 2015; Scheuer & Black 2000). Vascular invasion of the cartilage matrix via the periosteum initiates the formation of primitive marrow spaces and the primary marrow cavity, and introduces osteoprogenitor cells that differentiate into osteoblasts, which produce Type I collagen and mineralised matrix (Saladin 2015; Schoenwolf et al 2015). This process continues towards bone ends and represents the formation of the primary centre of ossification and early cancellous bone (Saladin 2015; Scheuer & Black 2004). The primary ossification centre represents the primary locus of bone formation for a given skeletal element that will eventually form an entire bone (e.g. sesamoids, carpals and tarsals other than the calcaneus) or the majority of an adult bone, which will in turn form from the fusion of multiple ossification centres (Scheuer & Black 2004).

Secondary ossification centres develop in anatomical regions that are related to the surface of joints, muscle or ligament attachment sites, where the primary centre of ossification does not develop to form an entire bone (Saladin 2015). Secondary ossification centres or epiphyses (when referring to long bones) develop at the proximal and distal ends of a given bone at various points during post-natal life (Saladin 2015). Epiphyses are separated from the diaphysis (primary ossification centre or long bone shaft) by a cartilaginous epiphyseal plate (see Figure 2.7). The epiphyseal plate facilitates longitudinal bone growth throughout development, and is made up of multiple zones that contain chondrocytes in various stages of development and

calcification (Saladin 2015; Scheuer & Black 2000, 2004) (see Figure 2.8). Fusion between primary and secondary ossification centres occurs when the rate of osseous matrix deposition exceeds that of cartilage proliferation and growth, and represents the cessation of longitudinal bone growth (Scheuer & Black 2004).

Figure 2.7. The process of endochondral ossification and primary and secondary ossification centre formation in a long bone. Taken from Scanlon & Sanders (2011).

Figure 2.8. The major components of a diaphyseal growth plate. Taken from Scheuer & Black (2004).

2.3.2. Properties of hyaline cartilage

Cartilage is a specialised avascular form of connective tissue that consists of chondrocytes embedded within an extracellular matrix (ECM) (Ovalle et al 2013). The functional and structural components of the ECM are produced and secreted by chondrocytes to form a complex structure that acts to support the overall integrity of the tissue and facilitate growth and development (Gentili & Cancedda 2009). The ECM is an amorphous, 'gel-like' substance that comprises a host of macromolecules that interact with one another to allow the tissue to resist strong tensile, shearing and compressive forces, and facilitate tissue growth (Gentili & Cancedda 2009; Ovalle et al 2013). Cartilaginous tissue is generally differentiated into three main types according to the composition of the ECM and the histological appearance of the tissue (Ovalle et al 2013). Hyaline cartilage provides a growth model for endochondral ossification, makes up the epiphyseal growth plate in long bones and lines the surface of synovial joints; elastic cartilage enables tissue flexibility and bending; and fibrocartilage facilitates attachment between joints and tendons, and even distribution of mechanical loading (Ovalle et al 2013). The structure and function of hyaline cartilage is considered in more detail given that the costal cartilages comprise this specific type of cartilaginous tissue. The degeneration of this tissue as a function of age is thus

important in estimating chronological age using the Isçan sternal rib end Phase aging method (see below).

The ECM of hyaline cartilage can be conceptualised as a mesh like network of Type II collagen fibrils interspersed with hydrated ground substance, that contain proteoglycans (PGs) and glycosaminoglycans (GAGs) specific to this type of cartilage (Gentili & Cancedda 2009; Roughley & Lee 1994) (see Figure 2.9). PGs are macromolecules comprised of a central protein core that is covalently bound to sulphated carbohydrate chains (GAGs); the function of PGs within the hyaline cartilage is largely determined by the overall structure of the PG/GAG complex (Gentili & Cancedda 2009; Roughley & Lee 1994). Hyaline cartilage contains at least five PGs within the ECM; the largest of these in size and relative abundance by weight is aggrecan (Gentili & Cancedda 2009; Roughley & Lee 1994). Aggrecan forms large PG aggregates within the ECM via interaction of the protein core with hyaluronan or hyaluron acid, a non-sulphated GAG (Gentili & Cancedda 2009; Knudson & Knudson 2001; Roughley & Lee 1994; Watanbe et al 1998a). In addition to hyaluronan, sulphated GAGs attached to aggrecan complexes draw large quantities of water into their structures to increase osmotic swelling and permit the hydration of cartilage, thereby enabling cartilaginous tissue to resist strong compressive forces (Gentili & Cancedda 2009; Roughley & Lee 1994). Common sulphated GAGs include chondroitin sulphate, dermatan sulphate, keratan sulphate and heparin sulphate (Gentili & Cancedda 2009). The collagenous ECM fibril framework structurally contains pressure resulting from osmotic swelling, providing resistance to tensile, shearing and compressive forces while maintaining the stiffness and elasticity of cartilaginous tissue (Gentili & Cancedda 2009; Watanbe et al 1998a).

Figure 2.9. Molecular structure of components of the extracellular matrix of collagen. Taken from Chen et al (2006).

2.3.3. The aging of hyaline cartilage

The ability of hyaline cartilage to withstand significant degeneration as a function of advancing age is in part due to the properties of the ECM and the proteoglycans within its matrix (Roughley & Lee 1994), although this ability is limited in mid- to late-adulthood where calcification is most significant (Dearden et al 1974). Possible explanations for the occurrence of age related degenerative changes to hyaline cartilage include variation in PG core protein gene expression and in the processing of enzymes that are responsible for the glycosylation and sulphation of GAGs, and proteolytic alteration of PGs following secretion into the ECM (Roughley & Mort 1986). Age related changes in the structure and composition of costal cartilages occur at the level of chondrocytes and the ECM.

Advanced age has been found to be associated with the degeneration and overall reduction in the abundance of chondrocytes (Dearden et al 1974). This process is characterised by the formation of vacuoles and lipid globules within chondrocytes and an amorphous, dense, granular halo around cells, in addition to increasing collagen fibril thickness and the occurrence of calcification agents such as apatite crystals (Dearden et al 1974). Degenerative effects at the component level of the ECM include:

a decrease in the size and content of PG complexes; increased levels of keratin sulphate in relation to chondroitin sulphate and proteins relative to GAGs; and diminished capacity of the core PG protein to interact with hyaluronic acid (Roughley & White, 1980). Collectively, these changes facilitate decreased cartilage elasticity and an increase in the degree of ossification and calcification (Rejtarova et al 2009).

2.4. The use of the sternal rib end in adult age estimation

The estimation of age in adult individuals is traditionally based on the morphoscopic analysis of patterns of skeletal degeneration and senescence that occur in physical bone specimens (Scheuer 2002). These estimates are inherently associated with larger error in comparison to age estimates for juvenile individuals as key developmental markers associated with the latter are no longer present, and the process of aging is varies within and between individuals and populations according to genetic and environmental factors (Franklin 2010; Garvin et al 2012). The estimation of adult age from the sternal rib has historically been associated with a considerable amount of debate, particularly regarding the overall accuracy and the legitimacy of the original method devised by Isçan et al (1984b, 1985; White et al 2012). Methodological reservations aside, the morphological metamorphosis of the sternal rib is recognised as an important source of biological age information that is utilised in contemporary practice (Garvin & Passalacqua 2012).

2.4.1. The sternal rib as an age marker

The utility of the sternal rib as an age marker was originally established using observation studies based on the direct examination of high resolution thoracic roentgenograms of cadavers at autopsy (e.g. McCormick 1980; McCormick, Stewart & Langford 1985; see also Figure 2.10). However, it was later widely acknowledged that the sole use of this method did not provide the level of precision necessary for analysis in a forensic context given individual variation between subjects (Barchilon et al 1996; McCormick 1980). Those seminal studies identified a general pattern of osteogenesis and ossification within the costal cartilages that correlated with advancing age, and manifested differentially according to the origin of the rib and sex of the individual (Scheuer & Black 2000; see Figure 2.11).

Figure 2.10. Examples of anterior chest roentgenographs of a 24-year-old man (pictured left) and an 80-year-old man (pictured right). Taken from McCormick & Stewart (1988).

Figure 2.11. Characteristic male (A) and female (B) patterns of ossification. Taken from Scheuer & Black (2000).

Ossification as a function of age has been shown to occur universally in a number of different populations, although the extent and pattern of ossification between groups has been demonstrated to vary according to dietary and endocrine factors (Semine & Damon 1970). In general, ossification of the costal cartilages begins during late adolescence and early adulthood, although the onset of the ossification process varies based on population affinity and sample demographics (McCormick & Stewart 1988; Rao & Pai 1988). Ossific nodules develop within subperichondral and central cartilage regions and typically appear in close proximity to the sternum or sternal rib ends, gradually increasing in size and coalescing with other nodules to produce extensive areas of ossified cartilage tissue that are characteristic of elderly individuals (McCormick & Stewart 1988).

This pattern of nodule formation and distribution is thought to be highly predictive of sex. The male pattern of ossification is characterised by the formation of subperichondral spurs that project along the superior and inferior rib margins that appear as intermittent ossifications during the late 20s and early 30s, and become continuous in individuals over 50 years old (McCormick & Stewart 1988). In contrast, the female pattern is characterised by cone shaped bone formations that appears centrally within the costal cartilage and extend anteriorly towards the sternum; in elderly individuals, these formations cause ribs to take on a 'crab claw' appearance (McCormick & Stewart 1988). A number of standards that attempt to estimate sex and age based on patterns of costal cartilage ossification have been established (e.g. McCormick & Stewart 1988; McCormick, Stewart & Langford 1985; Navani, Shah & Levy 1970; Rao & Pai 1988), although the Isçan et al method represents the first formal system for age estimation within a forensic context.

The method of forensic age estimation devised by Isçan (1984b, 1985) is based on the morphoscopic (or direct visual analysis) of the morphology of the fourth right sternal rib end, which undergoes metamorphosis as a function of advancing age (Isçan 1984b). Morphoscopic analysis is usually employed in the course of cranial based ancestry estimation, although the term is also used to refer to traits that are "non-pathological variations of skeletal tissues that can be better classified as present or absent (or as a point on a morphological gradient; e.g. small to large) rather than quantified by a

measurement” (Bukistra & Ubelaker 1996 in Hefner 2016, pp. 302). These traits can be further broken down into a series of classes, including those that describe the shape of bone, bone morphology, and the presence, absence or prominence of a given feature (Hefner 2016). Morphoscopic techniques are thought to offer a more rapid and convenient means of analysis (Isçan 1984b) and have been employed for the estimation of adult age based on the morphological appearance of skeletal elements such as the pubic symphysis (Todd 1920). The fourth right rib has traditionally been utilised as an age marker as it is thought to be less variable in its morphological expression compared to other skeletal elements, in that it is subjected to lower amounts of direct mechanical and functional stress, and is exempt from the influences of weight bearing, locomotion and parturition (Isçan et al 1991). As such, observable morphological changes in the appearance of the sternal end of the right rib are more likely to reflect the ‘true’ age of the individual (Loth et al 1994).

The Isçan method is based on the identification of degenerative morphological changes that alter the shape, form, texture and overall quality of bone at the fourth right sternal rib end; changes in the appearance of the articular surface, wall and rim of the rib end are particularly diagnostic of age (Isçan et al 1984b, 1985). General age related changes in the overall quality of bone include an increase in porosity (bone appears to be less dense), irregularity and sharpening of the rim, and deepening of the articular surface which results in the formation of a pit (Isçan et al 1984b, 1985). The sternal rib of a young individual (under 16 years of age) is described as generally being firm and very solid, with a flat articular surface, rounded edges and a regular rim (Isçan et al 1984b). Successive changes associated with advancing age include gradual deepening of the pit, scalloping of rim edges, thinning of rib walls, and the formation of sharp, bony projections and ‘windows’ within the walls (Isçan et al 1984b). Specific changes in the appearance of diagnostic bone components with increased age (based on standards devised for Caucasian male individuals by Isçan et al 1984b) (see Figure 2.12) are as follows:

i) Pit: the medial articular surface or pit is amorphous in shape in young individuals, but presents as an observable indentation with a flat or billowed surface. Successive changes include gradual deepening and widening of this indentation between anterior

and posterior rib walls, causing the pit to take on a V shape which gradually takes on a moderate to very wide U shape as the walls of the rim decrease in thickness. In the later stages of degeneration, the floor of the pit can be filled with irregular bony projections.

ii) Rib walls: the walls are initially thick, dense, and smooth in appearance with scalloped or slightly wavy, rounded regular edges. Thinning and sharpening of edges occurs as deepening of the pit commences. Advanced age is associated with extreme thinning and increase in the porosity and fragility of the rib walls, and the appearance of window formations.

iii) Rib margin: the rim of the sternal rib end is rounded and regular in shape in young individuals, and gradually acquires a wavy or scalloped appearance. Increasing age causes the edges to appear more irregular with no uniform scalloping present. This irregularity persists into old age where long bone projections replace any remaining scalloping.

Figure 2.12. Fourth sternal rib end specimens obtained from male individuals at autopsy. Taken from Isçan et al (1984b).

An unidentified individual is assigned to an age Phase according to the appearance of these features and direct comparison to associated type casts and reference photographs that depict the morphological features of each Phase, which is associated with a mean age, 95% confidence interval and a corresponding age range. Separate

standards have been devised for male and female individuals to account for differences in endocrine function and hormone production (Isçan et al 1985). These differences manifest as differential rates of Phase attainment and patterns of degeneration in sternal rib end components, although both standards attempt to classify morphological change into one of nine defined phases (Isçan et al 1984b, 1985). The original method has also been tested using culturally and geographically dissimilar populations to determine overall accuracy, and to evaluate whether the pattern of morphological change in the fourth sternal rib end follows the same progression in these populations (e.g. Isçan et al 1987; Oettle & Steyn 2000; Salem et al 2014; Yavuz et al 1998; see Chapter Three for a more detailed review).

Contemporaries of Isçan et al have called the fundamental methodology and sampling procedures of the original method into question, suggesting that it has been widely implemented without being challenged. Cited limitations include bias, poor reproducibility of results and transposition of the method to other populations, small sample size, moderate to high levels of inter- and intra- observer error, large age ranges and age mimicry (Fantom et al 2010; Hartnett 2010). Further, the use of this technique may not be appropriate in forensic or archaeological contexts where bone preservation is poor, or it is unclear which specimen represents the fourth right rib (White et al 2012). Given the continual use of the fourth right sternal rib end in contemporary age estimation (Garvin & Passalacqua 2012), the completion of independent validation studies is important to establishing the overall accuracy, transparency, reproducibility and validity of the original method in modern populations (see Chapter Three for reviews of validation studies).

2.5. The use of medical imaging modalities in a forensic context: The Virtopsy project

Contemporary forensic practice has benefitted greatly from the concurrent development and routine use of modern medical imaging technology. An increase in the use of imaging modalities in a forensic context was largely initiated by the Virtopsy (or virtual autopsy) project in 2001 (Bollinger & Thali 2015). This project is the result of a transdisciplinary collaboration between a number of forensic and non-forensic medical specialisations, whom aimed to supplement traditional autopsy methods using non-invasive cross sectional and reconstructive imaging techniques, such as

computerised tomography (CT), magnetic resonance imaging (MRI) and 3D photogrammetry (Thali et al 2005, 2007). The main impetus for the inception of this method was the need to modernise autopsy techniques to make observations at death less observer dependent, increase the availability of, and access to, evidence by digital means and ensure the precise documentation of forensic findings (Bollinger et al 2008). The purpose of the Virtopsy method is analogous to that of conventional autopsy, wherein post-mortem examination facilitates explanation of the cause of death, visualisation and reconstruction of injuries and/or injury patterns, and reporting of vital reactions and any relevant patho-morphologic findings (Dirnhofer & Thali 2009). The Virtopsy approach is also applicable to cases involving living individuals where the determination of the severity of an assault is necessary to the appropriate sentencing of an offender, or is required to visualise the extent of damage to internal structures where external evidence is not present (Bollinger et al 2008; Dirnhofer et al 2006).

The documentation of internal body structures in the context of Virtopsy is performed using CT and MRI modalities. CT techniques utilise the measurement of x-ray attenuation signals to produce digital reconstructions of slices through a given section of the body (Kalender 2009). These are used to visualise the skeletal system, osseous fractures and lesions, gas, fluid accumulations, calcifications and foreign bodies such as medical implants or bullets (Bollinger & Thali 2015). The use of CT imaging is particularly advantageous for the analysis of cranial blunt force or projectile trauma, as the technique preserves spatial relationships between fractures and skeletal fragments that may otherwise be lost (or take significant time to reconstruct) when the scalp is removed or rolled off the skull during a traditional autopsy (Bollinger & Thali 2015) (see Figure 2.13). Other advantages specifically associated with the use of CT imaging include rapid examination times and improved spatial and temporal resolution compared to conventional x-ray techniques (Kalender 2009), and identification and three dimensional depiction of the topography of foreign objects to facilitate extraction (Bollinger & Thali 2015).

Figure 2.13. Burst fractures of the calvarium at autopsy (left) and corresponding pattern of fractures visualised using a MSCT reconstruction prior to autopsy. Taken from Thali et al (2003c).

In contrast to CT imaging, MRI modalities are used for the visualisation of soft tissue injuries and neurological and non-neurological tissue trauma in living and deceased individuals (Bollinger & Thali 2015; Thali et al 2007). Internal imaging techniques have been used to establish cause of death in a variety of contexts, including gunshot and ballistic trauma analysis (Thali et al 2003d), identification of thermal trauma (Thali et al 2002), determination of cause of death in fatal accidents (Plattner et al 2003), examination of the appearance of decomposing tissues (Thali et al 2003c), and documentation of pathological findings related to drowning (Christe et al 2008; Dedouit et al 2007) and hanging (Bollinger et al 2005).

The advantages and disadvantages conferred by the Virtopsy approach are inherently associated with the mode of analysis implemented and the nature of digital data. Unlike traditional methods, virtual techniques are non-invasive and non-destructive to forensic evidence, can be used to visualise structures that may be difficult to access using conventional invasive procedures (Bollinger & Thali 2015), are time efficient (e.g. overcome the need for time consuming procedures such as maceration to visualise osseous material) (Dirnhofer & Thali 2009), and are comparatively less observer dependent as internal and external surface documentation procedures do not rely on subjective verbal or written descriptions (Dirnhofer et al 2006). Digitally acquired data

can be stored for extended periods of time and provides a source of evidence that can be easily accessed, retrieved, transferred and manipulated after the fact in medico-legal cases involving both deceased and living individuals (Thali et al 2003a, 2003b). Further, the use of radiological data permits examination of bodies that are affected by infections, toxic substances and other biohazards, in addition to those that are precluded from examination by traditional probative methods due to social, cultural or religious reasons (Thali et al 2007).

Despite their utility, these techniques are also associated with a number of disadvantages, and hence need to be supplemented with traditional examination procedures to ensure their efficacy (Dirnhofer & Thali 2009). Images of a given organ or structure are void of characteristics such as colour, smell and texture which are easily observed during invasive autopsy, and can be diagnostic of a particular pathological finding (Bollinger & Thali 2015). Advanced decomposition and some cases of natural cause of death pose challenges to examination using virtual methods (e.g. not possible to diagnose cause of death due to arrhythmogenic coronary artery disease using radiological methods alone), and the use of a Virtopsy approach is constrained by financial reasons concerning the availability and operating costs of imaging modalities (Bollinger & Thali 2015). It is also important to recognise the general lack of experience in radiological post-mortem data interpretation and recognition of visual information, as forensic radiology is not the equivalent of clinical radiology performed on a corpse; forensic radiologists may encounter material that is not encountered by clinical radiologists, such as gases resulting from decomposition and the presence of maggots or other insects (Bollinger & Thali 2015).

The feasibility of a Virtopsy approach using fleshed human remains was evaluated by Thali et al (2003b) using the same criteria that are of interest in a traditional forensic autopsy proceeding. That is, the reconstruction and explanation of cause of death, sequence and nature of injury and documentation of any relevant patho-morphological findings in tissues, organs and bones (see Figure 2.14). The validation study demonstrated that MRI and CT imaging was superior to traditional autopsy in cases of tissue, skeletal and cranial trauma, and the diagnosis of cause of death via aspiration, air embolism and subcutaneous emphysema. The application of traditional

methods was found to be preferable in cases involving the diagnosis of organ lesions, fat embolism and haemorrhage.

Figure 2.14. Magnetic resonance image of drowning fluid (left image; indicated by the arrows) within the paranasal sinuses. Aspiration of these fluids at autopsy (right image). Taken from Thali et al (2003b).

Of the 47 combined causes of death established during invasive autopsy, a total of 26 were established independently using imaging techniques. The feasibility of the use of imaging techniques was overall confirmed in that both approaches fulfilled traditional criteria and provided “excellent spatial resolution that sometimes even exceeds what is possible in clinical practice” (Thali et al 2003b:15). Collectively, Virtopsy techniques have independently established the cause of death in approximately 80% of autopsy cases (Dirnhofer & Thali 2009), and have been employed in a variety of contexts as a source of supplemental data where a correlation between radiological and invasive autopsy results was found (Thali et al 2003d). Given the advantages conferred by a virtual approach, the scope of application of these techniques has increased to include other forensic disciplines, including forensic anthropology.

2.6. The rise of virtual anthropology

The use of cross sectional imaging modalities in the field of virtual anthropology is largely derived from the use of these techniques in related disciplines such as palaeoanthropology and archaeology, where they are utilised as a means of examining fossilised material and ancient remains (Cesarani et al 2003; Franklin et al 2016).

Contemporary advancements in imaging technology, and an increased recognition of the potential utility of these techniques in a pathological and medico-legal context, has resulted in the use of imaging techniques during forensic casework and for the validation of existing and novel methods of skeletal analysis (Franklin et al 2016). The term 'virtual anthropology' is defined as the application of modern slice imaging techniques (predominantly CT) to anthropological analysis, primarily for the compilation of a biological profile through the examination of a virtual, three dimensional set of skeletal remains (Brough et al 2012; Dedouit et al 2015) (see Figures 2.15 and 2.16). The use of contemporary imaging methods such as CT (and to a lesser extent MRI) permits the examination of entire skeletal structures and augments data collected using conventional radiological techniques (Dedouit et al 2015).

Virtual skeletal reconstructions are advantageous because they represent a non-invasive and non-destructive method of data collection and analysis, eliminate the need for time consuming procedures (such as maceration), can be performed in living individuals for the purposes of age estimation, and provide a potential resource for the development (or improvement) of population specific reference standards (Brough et al 2015; Dedouit et al 2015; Grabherr et al 2009). A concordance of traditional osteometric measurements and parameters between 3D CT reconstructions and dry bone specimens has been empirically demonstrated (e.g. Franklin et al 2013b; Verhoff et al 2008; and see Figure 2.17), suggesting that imaging techniques provide a valuable source of supplemental skeletal data. Several validation studies concerning the application of traditional analytical methods to virtual skeletal reconstructions have also further established the efficacy of the method.

Figure 2.15. Virtual three dimensional reconstruction of the skeleton of an 11-year-old female used in the current study.

Figure 2.16. Virtual three dimensional reconstruction of the skeleton of a 23-year-old male used in the current study.

Figure 2.17. Example of a 3D MSCT reconstruction of the medial surface of the left pubic symphysis (a) and the dry bone specimen (b) of an elderly Caucasian female. Taken from Dedouit et al (2007).

The use of virtual skeletal remains requires validation given the relative novelty of the method. Grabherr et al (2009) conducted a multi-detector computerised tomography (MDCT) feasibility study for the purposes of estimating the sex and age of 22 documented deceased individuals using virtual reconstructions of the skull, shoulder, pubic symphysis and proximal femora. Traditional dry-bone methods were used for the estimation of age and sex from the selected skeletal elements (ectocranial sutures, pubic symphysis, sexually dimorphic features of the skull and pelvis). This study demonstrated that sex could be estimated using MDCT reconstructions of the skull and pelvis. Estimation of age using MDCT images was more difficult in comparison, although the difficulties experienced were similar to those encountered when estimating the age of physical bone specimens (e.g. under-estimating the age of young individuals and over-estimating the age of older individuals). The potential utility of a virtual approach to the anthropological analysis of deceased individuals was thus established. Traditional methods of skeletal analysis have since been applied to the construction of the biological profile for living individuals using medical imaging modalities such as CT, in order to improve reference standards and reconcile matters of a legal nature pertaining to age.

2.6.1. Virtual anthropology and the estimation of age in living individuals

The estimation of age in living individuals is a relatively novel practice in forensic anthropology that is becoming increasingly important for the reconciliation of legal matters that require a determination of culpability in circumstances where an individual does not possess legitimate documents to verify their age (Franklin et al 2015; Schmeling & Black 2010). These matters are either civil or criminal in nature and pertain to individuals of various ages depending on the relevant proceeding. Age estimates produced for living individuals tend to apply to those in the late stages of adolescence or early stages of adulthood (Franklin et al 2015), although age can also be estimated in young children that lack sufficient birth records (Schmeling & Black 2010), or elderly individuals that are seeking a retirement or old age pension (Schmeling et al 2007; Schmeling & Black 2010). Estimates of age in living individuals are made in order to determine the probability that the individual of interest has attained a legally significant age (Schmeling et al 2007). Common circumstances that require the estimation of age in living individuals includes matters concerning illegal immigrants, refugees or asylum seekers, cases involving human trafficking or child pornography, establishing legitimate age when falsification of birth records is suspected, and determining age bands for professional athletes (Franklin et al 2015; Schmeling & Black 2010).

Age estimates in the living are associated with a significant degree of legal and evidentiary value, because they contribute to the potential prosecution (or exoneration) of an individual and the determination of long term social outcomes (Franklin et al 2015; Ritz-Timme et al 2000). Inaccurate age estimates, or the inaccurate assessment of an individual as either a child or an adult, may result in a violation of human rights whereby the individual is detained in the wrong facility or is exposed to mistreatment or abuse from older individuals (Franklin et al 2015). As such, it is especially important to produce accurate estimates of age in individuals under the age of legal imputability. The age of criminal responsibility varies according to the jurisdiction in question, although it generally ranges from 7 to 22 years in most countries (Ritz-Timme et al 2000; Schmeling et al 2007, 2008).

Guidelines established by the Study Group on Forensic Age Diagnostics for the criminal prosecution of living individuals recommends an age estimate based on the results of a physical examination (collection of anthropometric data and degree of development of secondary sexual characteristics), x-ray examination of the left hand and dentition, and CT evaluation of the medial clavicle if bones in the hand-wrist region have reached complete maturity (Schmeling et al 2007). Methods used to estimate age in living individuals should be tested and acknowledged to be sufficiently accurate for medico-legal purposes, be based on non-invasive examination techniques, and comply with medical ethics (issues of confidentiality and informed consent) and legal considerations (Fernie & Payne-James 2010; Franklin & Flavel 2015, Franklin et al 2015; Schmeling et al 2007; Ritz-Timme et al 2000). The majority of living age estimates are made in juvenile individuals given the need to determine the probability of legal culpability.

2.6.2. Dental age estimates in living juveniles

The age of juvenile living individuals is generally estimated according to patterns of tooth development, eruption and mineralisation. Dental radiographs (or orthopantomograms) are compared to composite dental atlases or published developmental standards, and tooth development stages are scored to produce an overall maturity score that is then compared to reference data to estimate age (Franklin et al 2015). Commonly employed methods for the sex specific estimation of age based on the use of individual or multiple teeth include the Moorrees (1963a, 1963b) and Demirjian (1973) dental reference standards (see Chapter One).

The Demirjian method of age estimation is based on the rating of tooth developmental criteria for seven mandibular teeth, where a composite dental maturity score is derived from summing the scores of individual teeth, which are then converted to provide a final dental age. This method is applicable to individuals between 2 and 20 years of age, albeit it is known to overestimate chronological age in individuals of a different ancestral affinity to that of the original study population (Koshy & Tandon 1998; Tunc & Koyuturk 2008; Willems et al 2001), thus emphasising the need to use population specific reference data in order to enhance the accuracy of age estimates. In contrast, the Moorrees method has been shown to underestimate

chronological age in mixed South African (Phillips & van Wyk Kotze 2009) sub-adult populations. Dental age estimates in adolescents are largely limited to the analysis of the development of the third molar using either the Moorrees (1963a, 1963b) or Demirjian (1973) methods, although such methods are generally not employed as the development and eruption of this particular tooth is influenced by genetic factors (Franklin et al 2015) and ancestry (Olze et al 2004).

2.6.3. Skeletal age estimates in living juveniles

Skeletal age estimates are based on the evaluation of development relative to complete maturity. Common methods utilised for the forensic estimation of age in living individuals include those devised by Gruelich and Pyle (1959) and Tanner et al (1983). These standards were originally devised to study growth trajectories to determine the effect of factors that influence growth (nutrition and chronic disease), identify growth abnormalities, and diagnose growth and endocrinological disorders (Gruelich & Pyle 1959; Tanner et al 1983). The Gruelich and Pyle method relies on the comparative analysis of anterior-posterior radiographs of the left hand and wrist to a radiographic atlas containing data from male and female juvenile individuals between the ages of three months and 17 years. An age estimate is obtained by comparing maturity indicators (skeletal developmental milestones) of individual bones and their associated epiphyses to reference radiographs and written descriptions, and assigning a given age based on bones being at the same stage of development. The original method is associated with an accuracy rate between 0.6 and 1.1 years (Franklin et al 2015), although validation studies using other populations have found this rate to vary, with the method overestimating the age in Dutch (1.7 months and 3.3 months in girls and boys respectively) and Turkish individuals (0.17 – 1.1 years in girls and 0.88 – 0.98 years in boys under the age of 17) (Buker et al 2007; Van Rijn et al 2001).

The method established by Tanner et al (1962,1983) similarly utilises anterior-posterior radiographs of the left hand wrist complex, to produce an estimate of age based on the mathematical evaluation of a maximum of 20 bones (Franklin et al 2015). Three different methods utilising a variety of bone combinations (RUS or radius, ulna and short bones or metacarpals; carpals only; and combined RUS and carpal methods) are used to assign an overall maturity score that ranges from 0 to 1000, which is then

compared to sex specific centile curves to provide an estimate of skeletal maturity (or age) for each individual method (Franklin et al 2015; Tanner et al 1983). The use of TW3 RUS and TW2 carpal methods have produced the most accurate estimates of age in female and male individuals respectively in a Western Australian population (SEE ± 0.09 -3.46 years for females, and SEE ± 0.02 -3.42 years for males) (Maggio et al 2016).

Imaging modalities such as MRI and CT have more recently been utilised to supplement traditional radiographic techniques that examine the relative maturity of skeletal elements for the estimation of age in living individuals, particularly those within the age range associated with legal responsibility. CT evaluations are commonly performed to quantify the degree of epiphyseal fusion of the medial clavicle; the fusion of which is known to occur within the age range that is relevant for establishing criminal imputability, and thus tends to be used for individuals over the age of 18 years (Cunha et al 2009). Current standards are based on the analysis of plain chest radiographs, where the degree of ossification of the medial clavicle is rated on a five stage scale (ranging from absence of ossification to complete ossification with no epiphyseal scar present) associated with a chronological age range (Schmeling et al 2004). Schmeling et al (2004) found that the age of criminal culpability (over the age of 21) was unequivocally attained by all individuals assigned to Stage 5 (average minimum of 26.0 years of age for males, and 26.7 years of age for females). Individuals in Stage 4 were found to approach the age of criminal relevance (between the age of 21.3 and 30.9 years of age in males, and 30 to 30.9 years of age in females) although the authors make the assertion that the possibility of these individuals being under the age of 21 cannot be reliably denied. Hence, a minimum age of 26 years of age at Stage 5 of ossification is recommended to responsibly determine culpability (Franklin & Flavel 2015; Schmeling et al 2004). The use of the stage method has been validated in CT (Schulz et al 2005; Franklin and Flavel 2015) and MRI modalities (Hillewig et al 2011; Schmidt et al 2007). The use of CT based technology confers advantages over conventional radiographic techniques, in that it overcomes limitations traditionally associated with the use of plain chest x-rays (overlap between posterior and anterior images impedes the quantification of fusion) (Franklin et al 2015, Schmeling et al 2004).

MRI technology has also been used as an alternative visualisation method of skeletal sites that have previously been examined using conventional radiological methods, such as the wrist. The non-ionising and non-invasive nature of this modality is optimised to the examination of living individuals, and can be advantageous in circumstances where the use of ionising imaging methods is prohibited due to medical or ethical reasons (exposure to radiation regulated by ALARA principle, or As Low as is Reasonably Achievable) (Dedouit et al 2015; Hillewig et al 2011; Milenkovic et al 2014). The use of MRI technology in the course of age verification proceedings is particularly important in professional sporting environments, where the use of ionising technology is considered to be medically unjustifiable (Dvorak et al 2007). Dvorak et al (2007) developed a novel system for the grading of epiphyseal fusion in the left distal radius based on a sample of 14 to 19-year-old males of Swiss, Malaysian, Algerian and Argentinian ancestry. Changes in the radial epiphysis were quantified into one of six grades, ranging from unfused to completely fused. The rate of fusion was found to vary between groups, with the onset of fusion occurring between the age of 15.9 to 16.7 years, and being completed between 17.9 and 18.4 years of age. The results of this study establish the potential utility of using a MRI based method to distinguish adolescent individuals within a narrow age range (14 – 19 years).

2.6.4. Age estimation in living adults

Age estimates performed in living adult individuals are subject to similar difficulties encountered with the use of physical bone specimens. Adult methods are grounded in the assessment of skeletal or dental degeneration and senescence, and are hence considered to be overall less accurate given the increasing discrepancy between physiological and chronological age as an individual ages (Cunha et al 2009). Age estimates in living adults are generally performed to determine whether an individual has reached the age of retirement where they do not possess documents to verify their chronological age, as is commonly the case with foreign immigrants seeking old age pension payments (Cattaneo et al 2008; Schmeling et al 2007). Current methods are based on the examination of the dentition and are either invasive or non-invasive in nature.

Aspartic acid racemization from dental enamel and/or dentine provides an accurate, quantitative means of estimating age that can be used for living and deceased individuals in a variety of post-mortem contexts (Waite et al 1999). Dentine constitutes the core portion of each tooth and lies between the enamel (outer, mineralised tissue that encapsulates the crown) and the pulp cavity (innermost, soft tissue component) (White et al 2012). Racemization is a naturally occurring, temperature dependent chemical process, which converts active L form amino acids (protein units) to their D form counterparts over an extended period of time. This process causes an accumulation of D form amino acids with increasing age, and as such the ratio of D to L form amino acids can be used to estimate the age of a living individual (Helfman & Bada 1975; Ogino et al 1985; Yekkala et al 2006). Aspartic acid is most commonly utilised in racemization methods as it undergoes form conversion rapidly enough to be detected in archaeological and living specimens (Ritz-Timme & Collins 2002). The use of dentine is considered to be preferable to enamel as it contains a greater proportion of protein, and is less susceptible to contamination and alteration of the composition of amino acids due to the effects of attrition and dental caries (Helfman & Bada 1976). The accuracy of this method varies based on the correlation between L/D ratios and chronological age (Yekkala et al 2006). This is determined by choice of tooth and what portion of the tooth is used, and preference of dentin over enamel as a source of amino acids and *vice versa* (Waite et al 1999; Yekkala et al 2006). The stated accuracy of this method is between ± 3 and 4 years for dentine (Ogino et al 1985; Ohatani & Yamamoto 1992; Waite et al 1999) and ± 2 and 11 years for enamel (Ohatani & Yamamoto 1992).

The use of non-invasive dental age estimation procedures based on the examination of radiographs (or OPGs) mitigates potential ethical problems associated with the use of destructive techniques that require the extraction of teeth from living individuals (Kvaal et al 1995). These methods are based on the measurement of the pulp chamber relative to the deposition of secondary dentine. Dentine is continually deposited throughout life, thereby reducing the overall size of the pulp chamber and increasing internal tooth dimensions (Cameriere et al 2007; Kvaal et al 1995; Landa et al 2009). The quantification of this relationship provides a means of estimating chronological age. The Kvaal method (1995) is commonly used for the estimation of chronological

age in adults (living and deceased) and is based on the calculation of tooth ratios for six teeth using periapical radiographs. The standard error for the mean age estimate of this method ranges from ± 8.6 to 11.5 years depending on the number and type of teeth examined (mandibular versus maxillary and multiple versus singular teeth). This method relies on teeth being in normal, functional occlusion (free from pathological conditions such as dental caries, attrition, abrasion or trauma) and any significant dental reconstructive dental work such as fillings and crowns, making its application somewhat limited (Kvaal et al 1995). Virtual methods of skeletal analysis have been explored as a potential alternate source of data in cases requiring age estimation of the living.

2.7. Virtual techniques and adult age estimation

Adult age estimation methods rely on the morphoscopic evaluation of degenerating skeletal elements and the quantification of such as a function of age. Skeletal elements traditionally used for adult age estimation include the auricular surface (Buckberry & Chamberlain 2002), pubic symphysis (Brooks & Suchey 1990) and sternal rib end (Isçan et al 1984b, 1985) (see Chapter Three). The nature of virtual anthropology requires the transposition of traditional means of skeletal analysis (direct observation of dry bone specimens) to a virtual set of remains, in order to establish a biological profile. Skeletal elements that have historically been used for the estimation of age have been examined using virtual reconstructions (see below). Comparable results between dry bone and virtual skeletal samples have been empirically established (Bassed et al 2011b; Franklin and Flavel 2015a; Kellinghaus et al 2010; Schulz et al 2005), meaning that traditional techniques can be used to estimate age using virtual reconstructions of living and deceased individuals alike. Further, the use of virtual methods enables the creation and/or improvement of population specific skeletal reference standards, which are necessary to increase the accuracy with which tenets of the biological profile are estimated (Franklin et al 2016). The use of traditional (albeit virtual) based age estimation techniques can potentially supplement (or supersede) current age estimation methods for living adults that require the use of invasive or destructive procedures such as dentine aspartic acid racemization (Cattaneo et al 2008), or those that are insufficiently accurate, such as the assessment of dental attrition (Kvaal et al 1995). Current validation studies are based on the appraisal of virtual skeletal

reconstructions, although seldom utilise data obtained from living individuals given potential health risks associated with radiation exposure (Wink 2014). The main objective of current CT based studies is the establishment of sufficient visualisation of skeletal structures, from which biological age estimation analysis may be performed.

2.7.1. Auricular surface

The auricular surface of the ilium represents the medial articulation point of the ilium of the pelvis and the lateral aspect of the sacrum (sacroiliac joint) (White et al 2012). Age estimation from the auricular surface depends on the interpretation of the topography of ridges and grooves in the surface of the bone. Current methods are derived from the original and revised standards of Lovejoy et al (1985b) and Buckberry and Chamberlain (2002) respectively. Age related changes in the appearance of the auricular surface include a loss of defined billowing, fine surface granularity and transverse organisation, development of distinct striae, lipping of bony margins at the apex and destruction of subchondral bone (microporosity) (Lovejoy et al 1985b). Barrier et al (2009) evaluated the applicability of aspects of the auricular surface aging method devised by Lovejoy et al (1985b) and Buckberry and Chamberlain (2002) to CT reconstructions of os coxa. Macroscopic evaluation criteria were classed as being absent, moderate or marked, and included transverse organisation (appearance of radial striae/billows that pass between the medial and lateral borders of the auricular surface); macroporosity (diffuse or focal perforations in cortical bone that have a diameter of less than 1 millimetre); and apical changes (development of osteophytes at the margin of the apex, causing lipping) (Buckberry & Chamberlain 2002; Barrier et al 2009). CT reconstructions were shown to be useful in visualising macroscopic structures necessary for the estimation of age, although the spatial resolution of CT scans was identified as a potential problem for the visualisation of certain features (such as macroporosity). Transverse organisation was the most accurate feature for the estimation of age in adults under the age of 40 years, with almost perfect levels of inter- and intra- observer error ($K = 0.82$).

2.7.2. Pubic symphysis

The pubic symphysis is a joint located in the midline of the pubis that is formed laterally by the pubic bones of the pelvis and joined medially by a disc of fibrocartilage (Becker et al 2010). Methods used to classify morphological change as a function of age at this site rely on the recognition of the degree of wear in the symphyseal surface. The symphyseal morphology of a young adult is characterised by a series of pronounced, rugged horizontal ridges bordered by grooves in the bone, which become less distinct and pronounced with increasing age (White et al 2012). Several standards for the estimation of age from the pubic symphysis are available (e.g. Gilbert & McKern 1973; Meindl et al 1985; Todd 1920) and vary in Phase number and description of morphological criteria, sample size and demography. The Suchey-Brooks method (Brooks & Suchey 1990) is most commonly applied in a forensic context as it provides the highest correlation with chronological age (Lottering et al 2013).

Telmon et al (2005) conducted an inter-method study of the Suchey-Brooks approach in a sample of three dimensional CT reconstructions of the pubic symphysis, in addition to the physical bone specimens those reconstructions were constructed from. Seven different features of the symphyseal face were analysed, including bone texture, the morphology of ridges and the ventral rampart, depression of the symphyseal face, appearance of the symphyseal rim and ligamentous outgrowths, and delimitation of the two pubic extremities (see Figure 2.18). No significant difference in age estimates using either method was found to occur. Agreement between bone specimens and CT reconstructions in relation to the visualisation of symphyseal features ranged from 81% for the ventral rampart, to 100% for the appearance of ridges and extremities. As such, CT reconstructions were deemed a reliable supplemental source of data that can be used in place of physical bone specimens.

Figure 2.18. Dry bone specimen (left) and MSCT reconstruction (right) of the pubic symphysis assigned to Suchey-Brooks Phase I. Taken from Telmon et al (2005).

Wink (2014) retrospectively applied the Suchey-Brooks (1990) method to three dimensional pelvic CT reconstructions of living individuals to determine the accuracy of this technique in a clinical context. The age of the individual at the time of the CT scan was estimated correctly 79.5% of the time over two separate trials, with true age ranges (age range associated with each pelvic image that captured, or tended to over or under estimate the patient's age) being determined 70% and 87.5% of the time in male and female individuals respectively. Underestimation of the chronological age of individuals occurred in cases of misclassification, particularly in individuals assigned to Phase III and older. Superior visualisation of ossific nodules and the symphyseal rim was reported, and these features were most diagnostic of age in this sample. This study demonstrates that CT reconstructions are a valuable source for the visualisation of skeletal features in living individuals. However, given the clinical nature of the study protocol, the inter-method error and thus the overall accuracy of the use of CT reconstructions, could not be evaluated.

2.8. Virtual anthropology and disaster victim identification

The non-invasive and efficient nature of CT based imaging technology lends this technique to the examination and identification of human material in mass disaster scenarios. CT imaging techniques have been employed to augment current disaster victim identification (DVI) procedures to decrease the time required to collect all necessary post-mortem data relevant to establishing individual identity (Sidler et al 2007). This is particularly important in contexts where there are significant casualties, investigators have to contend with unfavourable weather conditions that may compromise forensic evidence, or investigators are not permitted to perform invasive autopsy procedures due to religious, cultural or social reasons (Blau et al 2008; Sidler et al 2007). Disaster scenarios involve multiple fatalities, with remains in various states of completeness and decomposition according to the nature of the disaster (e.g. flood, fire, explosion, bombing, terrorist attack), and the environmental conditions following the event (Blau et al 2008; Blau & Briggs 2011). The primary goal of the forensic anthropologist is the reconciliation and identification of human skeletal remains (O'Donnell et al 2011), although differentiation between human and non-human skeletal material, fragment analysis, assessment of the minimum number of individuals and identification of commingled remains may also be required (Blau & Briggs 2011). The incorporation of CT imaging in mass disaster scenarios has largely been associated with analysis of skeletal elements that may otherwise not be examinable due to the fragile condition of evidence.

The use of CT technology has a significant role in the identification of individuals involved in mass disaster scenarios in Australia. For example, CT imaging used in the course of a light plane crash enabled investigators to identify skeletal elements irrespective of their size or state of preservation in remains that were initially unrecognisable as human in origin, facilitating preservation of the remains, as the evidence did not have to be disturbed by a physical examination (see Figure 2.19) (Blau et al 2008). The process was overall found to be more efficient, as bones did not have to be macerated to be analysed, and identification of one individual was made based on the visualisation and identification of a medical implant (Blau et al 2008). CT imaging techniques have also proven advantageous in bushfire disaster scenarios, enabling anthropologists to differentiate between human and non-human skeletal

material, recognise commingling, and identify individuals based on the presence of primary and secondary centres of ossification and dentition (O'Donnell et al 2011). The identification of individuals based on skeletal remains and any associated objects (e.g. medical implants, personal items such as jewellery or prostheses) is particularly important to the investigative process in scenarios where traditional methods such as dental or DNA analysis are not applicable (O'Donnell et al 2011).

Figure 2.19. Superior view of unrecognisable human remains recovered from the crash site (above) and the same remains visualised as a MSCT reconstruction (below). The pelvis, sacrum, proximal right femur and selected vertebra can be observed in the reconstruction. Taken from Blau et al (2008).

2.9. The problem with estimating skeletal age

The estimation of age-at-death based on the physiological state of skeletal remains arguably represents one of the most significant challenges for a forensic anthropologist. Adult age estimation is considered to be particularly problematic given the heterogenous nature of the aging process (influenced by a number of genetic, behavioural and environmental variants), variability in available methods, and the level of methodological bias that is inherently associated with these methods (Kemkes-Grottenthaler 2002; Schmitt et al 2002). Traditional methods of age estimation rely on the use of documented skeletal collections for the creation of reference standards. Skeletal morphology is analysed and associated with a chronological age according to the standards of a given reference skeletal collection (Hoppa & Vaupel 2002). The use of a reference collection as a basis for a given age estimation method assumes that the age at death (biological age) reported for each set of remains is correct, and that the aging process is homogenous across all individuals (Usher 2002). Reference collections vary in their composition according to the sex, age and geographic and/or temporal origin of individuals, the availability of skeletal and/or dental elements, and the provenance of remains (Usher 2002). An ideal reference collection is one that is easily accessible and contains a comprehensive representation of the variation within a population according to age and sex distribution, ancestry, and the socioeconomic and health status of individuals (Usher 2002). Given the amount of variation in the composition of global osteological collections, no single collection is deemed to be ideal. As such, reference and study populations must be matched according to shared demographic criteria (Usher 2002). The use of reference collections in the course of contemporary adult age estimation has been critically examined in lieu of issues of methodological bias that underscore theoretical principles associated with the field of paleodemography.

2.9.1. Paleodemography and the use skeletal reference collections

The use of reference populations for the estimation of biological attributes is derived from the field of paleodemography, which is concerned with the identification of demographic characteristics of past archaeological populations (Hoppa 2002). It is argued that when demographic parameters such as sex or age are known (or can be estimated) the resulting population structure can be applied to past or future

populations to assess the significance of these parameters (Hoppa 2002; Howell 1986). This principle (known as biological uniformitarianism) underpins the use of aging techniques in that the pattern of age related change in contemporary populations is not considered to differ significantly from that observed in past populations (Hoppa 2002). The resulting implications for population structure include assuming that populations have not significantly changed in their biological response to the environment, and that development and senescence follow a universal pattern across populations, irrespective of temporal and geographic factors (Hoppa 2002). Critical examination of the underlying theoretical assumptions that inform the use of skeletal collections has established that these collections represent a source of error that must be acknowledged to make age estimates more reliable (Hoppa 2002; Usher 2002).

The use of documented skeletal collections for the development of reference standards is essential to the compilation of a biological profile for an unknown individual. However, such collections have been criticised as being out-dated and not representative of the contemporary populations to which those standards are applied, resulting in decreased accuracy in the estimated biological profile (Dirkmaat et al 2008). The main concern associated with the use of reference collections is age mimicry, or the tendency of skeletal age estimates in adults to mimic the structure of the reference collections upon which these standards are based (Bocquet-Appel & Masset 1982; Boldsen et al 2002). Further, there is a relative paucity in the number of studies that seek to evaluate the composition of skeletal reference collections to determine whether they are representative of the population from which they were drawn, and hence reliable in the formulation of standards (Usher 2002). Komar and Grivas (2008) identified a series of sample biases within the Maxwell collection and overall deemed it to be unrepresentative of the living population the specimens were drawn from. The latter biases included a general over representation of males and individuals over 45 years of age, those of Caucasian ancestry and individuals who suffered a traumatic cause of death. The authors concluded that while skeletal collections provide a valuable means of developing standards for the estimation of the biological profile, they are not a suitable proxy for the analysis of contemporary skeletal remains.

2.9.2. Methodological issues associated with age estimates

Currently, there is no universal consensus regarding the use of a particular aging method over another, the overall combination of data gained from multiple skeletal elements, and the application of certain statistical analysis procedures. This lack of standardisation makes forensic analysis somewhat arbitrary and dependent on the experience and discretion of the observer (Buckberry 2015). A survey by Garvin and Passalacqua (2012) into adult age estimation procedures implemented by forensic anthropologists found that most respondents tended to estimate age based on their own experience on a case-by-case basis, reporting both narrow and broad age ranges, and citing invalid or inappropriate statistical procedures as the reason for varying estimates. Multifactorial statistical approaches were employed by only 25.8% of respondents, despite evidence that suggests that the use of multifactorial methods increases the accuracy with which estimates are made, relative to the use of a single age indicator (Franklin 2010; Lovejoy et al 1985a). The Suchey-Brooks pubic symphysis method was ranked as the most popular for age estimation in adults, followed by the sternal rib (Isçan 1984b, 1985) and auricular surface (Buckberry and Chamberlain 2002; Meindel et al 1985). Although the authors do not provide a solution to the lack in standardisation, the anthropologist must remain cognizant of this issue in professional practice.

The methodological underpinnings of a particular technique must also be considered when estimating components of the biological profile. Sample size and distribution (among other factors) are especially important to results desired from a specific method, and the conclusions that are subsequently made about a broader population. Sample size influences the outcome of a study in relation to observing statistically significant relationships or interactions between variables, and thus ultimately influences the accuracy and quality of the study (Bartlett et al 2001). Small samples are generally considered to be disadvantageous as the resulting statistics are underpowered (containing low statistical power or the probability that the test will reject the null hypothesis when it is false). This means that the likelihood of observing a true effect is reduced, as is the possibility that a statistically significant result represents a true effect (Button et al 2013). Statistically underpowered studies are therefore associated with inconclusive results (low rates of result reproducibility and

overestimates of effect size) (Button et al 2013; Suresh & Chandrashekara 2012). Further, small samples are likely to be less representative of the population from which they are derived. This is problematic for the formulation of population specific standards because the resultant statistics are less accurate and less applicable. The above is also true of sample distribution; a significant sex-bias (discrepancy in the number of male and female individuals included in the sample) produces results that are skewed in favour of the better represented sex, thus limiting the ability to generalise conclusions to a wider population. The distribution of a sample may not necessarily reflect flawed sampling procedures, and may instead represent the composition of, for example, the skeletal collection that the sample was drawn from (skeletal collections may be associated with certain collection biases which cannot always be overcome).

2.9.3. The need for population specific data

Schmeling et al (2000) have suggested that skeletal maturation occurs at similar rates throughout geographically distinct populations and is more significantly influenced by socioeconomic and climatic factors. As such, the genetically predetermined growth potential of an individual is dependent on the availability of favourable environmental conditions such as access to medical care, nutrition and hygiene during prenatal and postnatal life (Gluckman & Hanson 2006; Delemarre-van de Waal 1993). Puberty represents one of the most rapid and dynamic periods of skeletal growth in humans, and is characterised by a growth spurt in early adolescence with concomitant sexually dimorphic alterations in body composition, size and shape (Delemarre-van de Waal 1993; Rogol et al 2002). The onset and rate of skeletal growth during puberty is mediated by a number of environmental signals. Factors that are known to reduce skeletal growth include (but are not limited to) poor hygiene, nutritional deficiencies or inadequate nutrition, chronic or acute illness and disease, high energy expenditure, emotional instability or chronic psychological stress, insufficient access to medical care, immigration and exposure to endocrine disrupting chemicals (interfere or alter the production, secretion and transport of hormones required for development, reproduction and homeostasis) (Fisher & Eugster 2014; Parent et al 2003; Schmeling et al 2000).

The retardation of skeletal growth during puberty is associated with diminished skeletal maturity in adulthood (Delemarre-van de Waal 1993). Population specific standards are therefore required to quantify the expression and magnitude of variation between and within populations. The application of either temporally or geographically foreign standards to a given population results in diminished accuracy in the estimation of the biological profile. As such, there is widespread acknowledgement within the community of forensic anthropologists that the use of contemporary reference data specific to a given population is necessary to maintain the accuracy and efficacy of a given analysis (Franklin et al 2013a; Lottering et al 2013).

2.9.4. Demands of age estimation in forensic practice

The medico-legal requirements of forensic casework inherently subjects the conclusions of a given analysis to judicial review and examination. In the United States, the admissibility of scientific forensic evidence by expert testimony is governed by the *Daubert* guidelines, which have a limited flow on effect on judicial procedure in Australia. The landmark *Daubert* ruling established that evidence should be tested using the scientific method, have been subject to peer review with established standards and known error rates, and be generally accepted as a relevant method within the associated scientific community (Grivas & Komar 2008). While these standards have not specifically been adopted in Australia, the amended Evidence Act of 1995 (Australian Law Reform 2012) permits the inclusion of expert opinion provided that it is based on specialised knowledge gained through experience, study or training (Section 79). Minimum standards for best practice within forensic anthropology established by the SWGANTH for the estimation of age include (if applicable) the use of population and sex specific standards, the recording and reporting of the error rates and evaluation of how measurements of error affect the reliability of results. The quantification and reporting of error is integral to the practise of forensic anthropology, as it ensures that methods are transparent, rigorous and repeatable, and produce results that are accurate and reliable.

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Chapter Three:

Current methods for the estimation of age based on the sternal rib

3.1. Introduction

The following chapter provides a chronological review of studies relating to the use of the sternal rib for adult age estimation. Traditionally, age estimation from the fourth right sternal rib has been performed through direct observation using a series of written descriptions, accompanying photographs and prototypic casts to describe and classify rib morphology according to either the Component or Phase method. Recent technological and methodological advances have facilitated revisions of the original method, thereby increasing its scope, efficacy and potential applicability in global forensic casework. The original Component and Phase methods are considered in detail, in addition to subsequent modified models that account for differences between the sexes and individuals of varying ancestry. Further validation studies using geographically and temporally disparate populations are also considered, given that they establish the accuracy and validity of the method. Contemporary adaptations and applications of the method using medical imaging technology and novel geometric morphometric techniques are also discussed, as they provide statistical quantification for the estimation of age in living individuals, and a platform for the development of contemporary, population specific standards.

3.2. The Component compared to the Phase approach

The Phase method for the estimation of age from the fourth sternal rib end owes its use in contemporary anthropological analysis to the earlier components based approach. The Component method relies on the identification and scoring of sequential changes that occur in three characteristic features (or components) of the sternal extremity as a function of advancing age. The subsequent Phase approach largely retains the use of the same features, although it groups age related changes into a series of discrete stages or Phases that are defined by a particular appearance of features, rather than a numerical scoring system. The following section examines the development of the Phase aging approach from its origins as a Components based method.

3.2.1. The Component method

The Component method devised by Isçan et al (1984a) represents the first attempt to establish a formal set of standards for the estimation of age based on the analysis of the sternal rib end. The inception of this method was influenced by previous radiographic and gross examination studies that established a linear relationship between advancing age and the degree of costochondral cartilage mineralisation that begins at approximately 15 years of age (McCormick & Stewart 1980; Semine and Damon 1975). The Component methodology of Isçan et al (1984a) is modelled on a similar system developed by McKern and Stewart for the estimation of age from the pubic symphysis (1957), and is claimed to provide a comparatively more accurate representation of the 'true' biological age of an individual since the morphology of the rib is not affected by pregnancy, parturition and locomotion (Isçan et al 1985).

The Component method relies on the examination of three skeletal characteristics (or components) of the right fourth sternal rib end; these include pit depth, pit shape, and rim and wall configuration (see Figures 3.1 to 3.3). Pit depth refers to the maximum dimension of the cavity that forms in the medial articular surface of the rib with age, and is defined as the distance between the bottom of the pit and the greatest height of an adjacent rib wall (measured using callipers). Pit shape refers to progressive changes in the appearance of the rib pit, which initially present as a shapeless, superficial indentation that gradually forms a V-shaped structure, which eventually takes on the appearance of a widened U-shape as thinning of the pit walls progresses. The final component describes changes in the configuration of the rim wall and pit, wherein there is a general loss of bone density, thinning of rib walls, and an increase in the irregularity of the rim (Isçan et al 1984a). Alterations in the morphological appearance of each component as a function of age related metamorphosis is defined by a series of either five or six stages. Each stage is associated with a mean age, standard deviation, 95% confidence interval and age range. An estimate of age is obtained by summing the composite score for all three components. The efficacy of applying a Components approach in a sample of Caucasian male individuals is considered below.

Figure 3.1. Component I (pit depth). Stage 0 is characterised by billowing of the articular surface with no pit formation. Stage 1; pit formation to a maximum depth of 1.6mm. Stage 2; Pit depth has increased to 3.7mm. Stage 3; pit depth is at 6.1mm. Stage 4; pit depth has reached 7.4mm. Stage 5; pit depth has reached 11.1 mm (adapted from Isçan et al 1984a).

Figure 3.2. Component II (pit shape). Stage 0 is characterised by no pit formation and a flat and billowing articular surface. Stage 1; initial formation of the pit (amorphous indentation) between the anterior and posterior rib walls. Stage 2; the forming pit takes on a V-shape. Stage 3; the pit takes on a narrow U-shape and has fairly thick walls. Stage 4; the pit is U shaped with thinning walls. Stage 5; the pit is a wide U shape with brittle and very thin walls, with some disintegration of bone (adapted from Isçan et al 1984a).

Figure 3.3. Component III (rim and wall configurations). Stage 0; the rim is smooth and rounded with no wall formation. Stage 1; the rim is still smooth and rounded with minimal wall formation that defines the shallow pit. Stage 2; rib walls are thick and dense with smooth surfaces and a scalloped rim. Stage 3; rim scalloping is less defined and the rim is becoming more irregular. Rib walls are thinning but remain fairly dense. Stage 4; the rim is becoming sharper and more irregular. The walls are thinner, less dense and increasingly deteriorated in texture. Stage 5; the rim is very sharp and irregular with bony projections. The walls are very thin, porous and brittle (adapted from Isçan et al 1984a).

3.2.2. Isçan, Loth and Wright (1984a)

This study aimed to develop a novel age estimation technique based on the appearance of the sternal extremity of the right fourth rib in a sample of individuals of known age, sex and ancestry (93 specimens obtained at autopsy representing Caucasian males between 17 and 70+ years of age). The composition of the rim and wall of rib ends was found to be the most accurate indicator of age related metamorphosis (eta squared 0.70), followed by pit shape (eta squared 0.68), and depth (eta squared 0.41). The combination of all three Components accounted for 73% of variation attributed to age related morphological change. Overall, pit shape, and wall and rim configuration were more reliable in estimating age compared to the use of pit depth alone (mean age increasing between 5 and 8 years in stages 1 to 3 and 10 years in stages 1 to 4 respectively). Levels of inter- and intra-observer error associated with this method were not evaluated. The stated overall accuracy rate (total Component scores) of this method is 16.81 (standard deviation) with a 95% confidence interval of 33.8 – 41.0, and an age range of between 17 and 76 years. The authors acknowledge the need to recognise factors that may affect the pattern of sternal rib aging and thus the reliability of the method (e.g. human variation, sex differences and health status), although conclude that the method provides a reliable alternative to other skeletal aging techniques.

This study suffers from a very small sample, meaning that there is insufficient data to develop a set of standards, or generalise findings to a broader Caucasian male population. There is a limited number of individuals in each age interval, and individuals between the age of 20 to 49 are over represented compared to other age groups (60% of the total sample). Further, the results have not been validated using larger and more diverse sample population. Finally, the lack of acknowledgement of levels of intra- and inter-observer error undermine the overall legitimacy of the study (see section 3.3 below).

3.2.3. The Phase method

The adoption of the Phase method follows the acknowledgment of Isçan et al that this technique is more practical, rapid and provides greater ease of use in comparison to the preliminary Components method (Isçan et al 1984b; see above). As such, the use

of the Phase ageing method has largely superseded that of the Component technique. The Phase method relies on the classification of fourth right rib specimens into one of nine Phases based on age related changes in the shape, form, texture and overall quality of bone at the costochondral junction. This method retains the use of skeletal features utilised in the Component method (the depth and shape of the rib pit, rim and wall configurations), although it eliminates the need to measure pit depth, and restricts pit depth descriptions to subjective characterisations of the degree of deepening from one Phase to another (e.g. “there is little change in pit depth, “the pit is noticeably deep,” and “the pit is very deep” 1984b:1099), instead placing greater emphasis on the shape of this structure (see Chapter Four for a detailed account of the method). Separate standards have been developed for male and female individuals to account for sex specific variation in skeletal morphology. The efficacy of the Phase method in a sample of Caucasian male and female individuals respectively is considered below.

3.2.4. Isçan, Loth and Wright (1984b)

This study aimed to develop a Phase age estimation method based on the analysis of the right fourth sternal rib end to increase the efficiency of adult age estimation techniques, and replicate estimates obtained from other Phase based methods using alternate skeletal elements, (such as the pubic symphysis; McKern & Stewart 1957). The sample comprised of 118 specimens obtained from Caucasian males between 17 and 70+ years of age at autopsy. Each rib was assigned to a given Phase (0 – 8) based on the analysis of pit depth and shape, and the appearance of the rib rim and wall.

An overall association between increasing age and metamorphosis of the sternal rib was established (differences between Phases statistically significant at $p < 0.001$), with the greatest rate of change observed to occur between Phases 1 and 4. Morphological change occurred at a slower rate between Phases 5 through 8, where increased variability in the appearance of rib features was observed. Individuals between 20 and 49 years of age exhibited the greatest variability in the appearance of rib features. A total 85% of the variance observed between specimens was associated with increasing age. The accuracy rate of this method is 7.51 years (standard deviation) with a 95% confidence interval of 39.6 to 42.4, and a range between 17 and 85, years of age.

Levels of inter- and intra-observer error were not reported. The authors concluded that the statistical analysis provided evidence of a causal relationship between age and Phase membership, thus confirming the suitability of the fourth sternal rib as an appropriate skeletal age marker. Sources of potential error such as observer experience, health status, occupation, population affinity and individual variation were acknowledged by the authors.

This study has similar limitations to those associated with the Components method. The distribution of specimens included in the sample is skewed in favour of those representing individuals between 20 and 49 years of age (approximately 58% of the sample), and is limited to only 6 individuals between 17 and 19 years of age. This is a significant limitation as the interval from 17 to 19 years of age falls within the transition from Phase 1 to 2, which encompasses the distinction between juvenile and adult status. This distinction is particularly pertinent to establishing culpability in legal proceedings. The unequal distribution of the sample over a broad age range (method stated to be applicable to those between 17 and 85 years of age) and a small sample severely limits the conclusions that can be drawn in relation to a broader population. The method becomes less accurate and less useful in estimating the age of individuals in later Phases as the age range cited for each Phase increases significantly. This begins to occur in Phase 5 where the age range spans a difference of 24 years, and increases to 41 years at Phase 8.

3.2.5. Isçan, Loth and Wright (1985)

This study aimed to devise a novel set of age estimation standards based on the Phase analysis of fourth right rib specimens derived from a sample of Caucasian females (86 sternal rib end specimens collected from individuals between 14 and 90 years of age at autopsy). The same analytical procedures established for the male individuals (Isçan et al 1984b) were followed in this study. Evidence of initial metamorphosis was observed earlier in the female sample (14 years of age), with pronounced change occurring by 16 years of age (morphology characteristic of Phase 2). The most rapid rate of morphological change was between the ages of 14 and 28, with considerably slower change occurring following this interval. A significant difference between Phases as a function of age was observed ($p < 0.001$). A total of 76% of the variance observed

between specimens was associated with increasing age. This rate is lower than that reported for males (85%; Iscan et al 1984b), meaning that the model was less effective for estimating age in females due to increased morphological variation. Variation in Phase estimation was greatest for individuals in their 20s, 40s and 70s at time of death.

Female specimens exhibited an altered pattern of metamorphosis compared to males, displaying development of a central arc within the anterior and/or posterior walls of the rib, with the formation of bony projections from precursor plaque deposits on the pit floor. In contrast, the generalised male pattern of metamorphosis was characterised by the formation of similar projections along the superior and inferior margins of the rib, with no central arc development. Females exhibited delayed growth of marginal projections in Phase 7 (mean age approximately 65 years) compared to males, where these projections were noted in Phase 5 and above (appearing at a mean age of approximately 39 years). Other differences include pit shape and the extent and timing of pit deepening. Levels of intra- and inter- observer error were not quantified. The authors concluded that the method for aging female ribs appears to parallel that for males. Similar limitations and further considerations were acknowledged, including the need to determine the sex of a set of skeletal remains prior to age estimation, and to be cognizant of individualistic factors that may influence variation, such as disease, occupation and nutrition.

The conclusions that can be drawn from the Iscan et al (1985) study are significantly limited by the unequal distribution of specimens across age intervals, and a small sample overall. The results of Phase 1 are based entirely on the appearance of a single rib specimen, meaning that no legitimate, statistically significant conclusions can be made considering the gamut of human variation. Similarly, only extremely limited conclusions can be made in regards to the statistics presented for Phases 2 and 3, given that the author's conclusions are based on the observations of five specimens. Individuals between 20 and 49 years of age are over represented in relation to other age groups. The utility of the method in providing an age range decreases from Phase 5 and onwards, where the range spans 48 years in Phase 5 to 28 years in Phase 8. The

lack of error quantification (see section 3.3 for significance) further diminishes the validity of the study.

3.3. Quantification of inter-observer error

The quantification and reporting of error is integral to the practice of forensic anthropology given the evidentiary and legal significance of results, as it provides an overall indication of the accuracy, validity and reliability of a particular technique or method (Christensen & Crowder 2009). Recent developments in evidentiary standards have established that a method must be associated with an appropriate set of standards, have documented error rates, be peer reviewed and accepted as a legitimate method by the forensic community (Christensen & Crowder 2009). Further, the Scientific Working Group for Forensic Anthropology (SWGANTH) recommends the recording and reporting of error rates, and the evaluation of the effect of error on the reliability of results. Failure to conduct precision tests and report error rates thus undermines the accuracy and legitimacy of results and the method applied.

3.3.1. Isçan & Loth (1986a)

The aim of this study was to evaluate the effectiveness of the authors' male age estimation standards (Isçan et al 1984b) in a sample of specimens of unknown age to determine the level of inter-observer error associated with this method. A sample of 15 fourth right rib specimens obtained from Caucasian males was presented to 25 individuals attending the 1984 Annual Meeting of the American Academy of Forensic Sciences (AAFS). These individuals varied in the level of their expertise, ranging from undergraduate students to those with extensive experience in either forensic or physical anthropology or forensic medicine. Individuals were asked to assign each of the unknown ribs to a given Phase by comparing the physical bone specimen (rib end only) to a series of photographic plates, depicting three prototypic ribs at each of the 9 Phases (0 – 8). Descriptions of rib morphology that ordinarily accompany each Phase photograph series were not provided (reasons for this decision were not cited). The authors followed the same method to estimate each rib to a given Phase. The actual age of each specimen was obtained from the records of the medical examiner to construct an 'ideal Phase' (the one "in which the specimen's age came closest to the calculated mean age per Phase lying within the 95% confidence interval" (1986a:123)).

The results of the inter-observer test were then compared to the ideal Phase. Finally, the authors correlated the relative experience of judging individuals with the effectiveness (accuracy) of the method to determine the degree of ease of application of the method.

Age tended to be underestimated across examiners when a deviation from the ideal Phase occurred. On average, 15 of the 25 judges aged specimens to one Phase within the ideal. For 9 of the 15 ribs evaluated, 80% of the estimates fell within one Phase. The mean deviation (calculated as the raw total of Phase deviations divided by the number of judges per case) for judges with doctoral degrees was 0.92, and 1.06 for those without doctoral degrees. The authors achieved a mean of 0.47. The overall deviation across participants was 0.97. The level of inter-observer error is classified as being minimal by the authors, although there is no numerical quantification of this. The authors concluded that the method can be used to estimate age to within one Phase of the actual age of the individual, and that the experience of the observer does not significantly affect the accuracy of this estimation.

The main limitation of this study is that Phase descriptions outlining the morphology of rib ends as employed in the original study (Isçan et al 1984b) were not used. This study is therefore not an exact replication of the original method and thus the level of inter-observer error determined cannot be readily applied to assess the effectiveness of the original method (Isçan et al 1984b). Descriptions of bone texture and the relative sharpness of edges are acknowledged by authors as important to the estimation of age, especially in older individuals. The study could also be improved by increasing the sample of test ribs to include multiple specimens from the same Phase to control for any variation that may occur between specimens of approximately the same age.

3.3.2. Isçan & Loth (1986b)

This study aimed to evaluate the level of inter-observer error associated with the use of the female Phase aging standards devised by Isçan et al (1985) on a sample of rib specimens of unknown age, in addition to comparing these results with previous male data (Isçan & Loth 1986a) to assess the effect of previous knowledge on the accuracy of age estimation. The method for this study follows that established by Isçan & Loth

(1986a; and see above). A total of 10 Caucasian female fourth right rib specimens were presented to 28 individuals attending the 1985 annual meeting of the American Academy of Forensic Sciences (AAFS). The method for age estimation in this study, and the range of experience of judging individuals, is similar to that established by previous work (Isçan & Loth 1986a). The female test differed from the previous male experiment in that the test ribs derived from the original Phase method were included and two control ribs (prototype bones for Phases 1 and 4 pictured in photographic plates used in the current study) were used to account for perceptual differences. A total of eight judges from the earlier male test (Isçan & Loth 1986a) were employed in the current study, and their scores were compared across the two tests and to the scores obtained from the remaining judging individuals in the current study. A single judge performed the test twice, once prior to the presentation of the original method by authors, and once following the presentation. All other judges performed the test prior to the presentation of the original method.

The female results were similar to those reported for males; age tended to be under estimated when a deviation from the ideal Phase occurred. Individuals without doctoral degrees estimated age with a lower level of accuracy compared to those with a doctoral degree (0.72 and 0.85 respectively). The authors achieved a lower mean deviation compared to that reported in the male test (0.20 to 0.47 respectively). The overall mean deviation across participants was 0.82, compared to 0.97 in the male test (Isçan & Loth 1986a). The prototype ribs were not assessed with a level of accuracy expected by the authors, yielding a mean deviation of 0.9 and 0.5 across all participants for each respective rib. Familiarity with the method increased the accuracy with which age estimates could be made, with the individual undertaking repeated assessments scoring a deviation of 1.6 prior to, and 0.2 following, the presentation of the original method. No numerical quantification of the total inter-observer level was provided. The authors concluded that the overall result supported the hypothesis that the use of photographic plates in age estimation is sufficient regardless of the level of experience of the observer, although acknowledge that the method could be improved with the use of osteological descriptions for each Phase.

The results of this study emphasise the need for access to accompanying osteological descriptions for each Phase to support the overall analysis given the perceptual differences (enlarged photograph to illustrate rib morphology compared to relatively smaller rib specimen) that are noted to occur as a potential source of error by the authors. The stated 'accuracy' of this method is not a true reflection of the accuracy of the original study (Isçan et al 1985) given that Phase descriptions were not utilised to estimate age. Overall, the study suffers from a small sample and unequal representation of Phases in the ribs that were presented for age estimation. This limits the overall accuracy of the error estimates that were provided, and the age ranges that can be associated with an estimate of error.

3.4. Validation of rib age estimation in other global populations

Human variation is the result of phenotypic adaptation to different environments and the influence of population specific cultural practices and factors. Body shape and size (in particular) is mediated by the complex interaction between genes and environment, and thus varies significantly between and within populations (Stinson 2000). Human growth and the final expression of body size and shape is also influenced by extrinsic factors, such as nutrition, health and socioeconomic status, disease, occupation, secular trends and hormone function (Scheuer 2002; Stinson 2000). Similarly, degenerative physiological and morphological changes associated with senescence have been shown to vary in their rate and degree of progression according to ancestry (Schmitt et al 2002). Contemporary population specific reference standards are, therefore, required to capture the expression and magnitude of variation between and within populations, in order to increase the accuracy with which age estimates are made. The following studies tested the efficacy of the original fourth sternal rib Phase method (Isçan et al 1984b, 1985) with culturally and geographically dissimilar populations, to determine the overall accuracy of the method, and whether or not the pattern of morphological change in the fourth sternal rib follows the same progression in those populations.

3.4.1. Isçan, Loth & Wright (1987)

The primary aim of this study was to determine whether the sternal rib of African American individuals follows the same pattern and rate of age related morphological change to that in Caucasian American individuals. A secondary aim was to develop a series of standards specific to an African American population, should ancestral differences be significant enough to warrant such modifications. The sternal end of the right fourth rib was removed from 63 African American individuals (49 males and 14 females) of known sex, age and ancestry at autopsy. Age was estimated using the Caucasian Isçan et al Phase aging method (1984b,1985) without prior knowledge of the age of each specimen. Phase examples are shown in Figures 3.4 to 3.6.

Figure 3.4. Pattern of morphological change seen in African American male ribs (Phase 1 to 3). Phase 1 development (initial pit formation and billowing of the articular surface) in a 15-year-old male (plates 1a – 1c). These changes do not occur until 17 years of age in Caucasians. Phase 2 scalloping distinctive of African American individuals (plates 2a – 2c); sometimes scallops are completely absent in individuals who exhibit all other characteristics of this Phase. Phase 3 (plates 3a – 3c) characteristics can range from typical, regular pattern of scalloping seen in Caucasian individuals, to the smooth and regular rim seen in plate 3b. The interior of the pit is similar to that observed in Caucasians (adapted from Isçan et al 1987).

Figure 3.5. Pattern of morphological change seen in African American male ribs (Phase 4 to 7). This is characterised by a pointed anterior or posterior rim (plates 4a – 4c) and a flattened rib configuration (plate 5a) in Phases 4 and 5. Rim irregularities begin to occur at Phase 6 (plates 6a – 6c). Pronounced projections from the superior and inferior rib margins appear during Phase 7 (plates 7a – 7c); the pit is porous but the bone remains firm (adapted from Isçan et al 1987).

Figure 3.6. Pattern of morphological change observed in African American female ribs (Phases 3 to 6). Rib exhibit a flattened configuration compared to Caucasian ribs throughout Phases. The pit is comparatively more U shaped and the interior contains more plaque deposits (plate 4c). Loss of bone density characteristic of Phase 6 is less pronounced in African American individuals compared to Caucasian individuals (adapted from Isçan et al 1987).

The mean age per Phase was reported as “nearly identical” for males up to Phase 5, and up to Phases 3 and 4 for females. Specimens collected from African American individuals were on average younger than their Caucasian counterparts, ranging from 7 to 10 years in males across Phases 6 and 7, and 3 and 6 years in females across Phases 5 and 6 (actual age). With the exception of Phases 3 and 4, African American ribs were consistently judged as being younger, particularly in the late 20s and onwards. The Phase aging method was found to account for 70% and 51% of age related change in African American males and females respectively. These figures are lower than those reported for Caucasian individuals (85% for males and 76% for females; Isçan et al 1984b, 1985).

General morphological differences observed between the two groups include earlier skeletal maturation in African Americans (initial pit formation noted at 15 years of age in a male specimen, compared to 17 years in the male Caucasian sample); less pronounced, regular or completely absent evidence of scalloping in the late teens and early 20s in this group; pointy, square or flattened rim configurations occurring during the early 30s in African Americans characteristic of Phase 7 or 8 in Caucasian ribs; and prominent extension of superior or inferior rim projections in the 30s, with increased frequency and rate of growth in the 40s (projections initially develop in the 40s in Caucasian individuals). Further, the sternal rib in African Americans was observed to maintain superior bone density and texture when compared to Caucasian individuals of the same age. Given statistical and morphological discrepancies, the authors concluded that differences in both the rate and pattern of sternal rib end metamorphosis do exist between Caucasian and African American individuals. As such, modifications of the original Caucasian standards for use in African American populations were recommended. Acknowledged limitations of the study include a potential selection bias across individuals (favouring those of a traumatic cause of death) and lack of documentation of cultural lifestyle factors.

The conclusions that can be drawn from this study are severely limited by a small sample with a significant sex-bias (49 males compared to only 14 females). This has resulted in a lack of data for female Phases 1,2 7 and 8 and male Phase 8, meaning that the method cannot be used to estimate the age of individuals within these Phases, thus limiting its overall scope and applicability. Given insufficient data to support conclusions regarding the appearance of the rib during these Phases, African American standards of morphological change cannot be compared to those established for Caucasian individuals, as performed by the authors. The use of Caucasian standards (Isçan et al 1984b) on African American individuals is arguably necessary to the establishment of differences that occur between the two populations, however the authors do not provide a set of descriptions for each Phase specific to African Americans, despite recommending modifications of the original method given observed differences in the rate and pattern of morphological change between the two populations. The use of population specific standards is widely acknowledged as

being important to ensuring the accuracy and efficacy of a given analysis (Franklin et al 2013a; Lottering et al 2013) (see section 2.9.3).

3.4.2. Yavuz, Isçan & Cologlu (1998)

The purpose of this study was to determine the efficacy of the sternal rib Phase aging method in a Turkish population, to establish whether Turkish ribs follow a similar pattern of age related metamorphosis to Caucasian ribs. This study utilised a sample of right fourth sternal ribs collected from 294 individuals (150 males and 144 females, ranging from 8 to 88 years of age) at autopsy. Each rib was estimated to an age Phase according to photographs and descriptions provided in the original Isçan et al studies (1984b, 1985).

Differences between Phases across male and female ribs were significant ($p < 0.001$). The amount of variance explained by the model (morphological appearance of features at each stage) was 86% for males and 84% for females in this population, compared to 85% and 76% for males and females respectively in the original American population (Isçan et al 1984b, 1985). In general, Turkish females were found to attain each Phase later compared to American females, and the same was found to be true for male individuals up to Phase 5. Variability in the appearance of rib features was found to increase following Phase 5 in both populations (measured by the standard deviation of each Phase; 11.37 for the Turkish population and 12.22 for the American population). Given the degree of similarity in the pattern of morphological change between populations, the authors concluded that the application of American standards is sufficient for age estimation in a Turkish population.

This study suffers from under representation of individuals at either end of the Phase age range. This is especially true for elderly individuals, where observations of males between 70 and 79 years of age are based on a single individual, and females in the 80 to 89 age group are not represented at all. Sound, statistically robust conclusions concerning the applicability of the Isçan method to a Turkish population therefore cannot be made for individuals within this age range, undermining the authors conclusions that American standards are sufficient for age estimation in a Turkish population.

3.4.3. Oettle & Steyn (2000)

This study applied Isçan et al standard (1984b, 1985) to a larger sample of South African Blacks, given inconsistencies identified in previous samples of African American descent (Isçan et al 1987). A total of 339 sternal ribs (265 males and 74 females, ranging from 0 to 99 years of age) were collected from the Pretoria State Mortuary and cadaver collection from individuals of known age, sex and ancestry. In addition to Phase analysis, a McNemar test for symmetry was performed to identify any bias in the direction of Phase analysis (under or overestimation of age, or lack of association with actual Phases). Age estimations were performed by three individuals who were familiar with the Isçan et al method (1984b, 1985); these were in concordance approximately 80% of the time (no other observer error reported). Comparison between the actual age Phases of each rib and consensus values reached by the assessors indicated that male ribs were under-aged in younger Phases, and over-aged in older Phases. Overall, male ribs were scored within one Phase of the actual age approximately 79% of the time, and scores assigned by the judges were in agreement in approximately 36% of estimations. In contrast, female ribs did not display a general trend for being under or over-aged at any given Phase. Female ribs were scored within one Phase of the actual age of the individual approximately 71% of the time, and scores between assessors agreed in 35.14% of cases. Given the poor level of agreement, the authors devised a novel set of standards for this population (altered Phase descriptions, although no statistics were provided).

This study is associated with a significant sex-bias in the sample population (265 males compared to 74 females), meaning that conclusions concerning female individuals are not necessarily sufficiently statistically robust and thus cannot be readily generalised to a Black female South African population. Further, no statistics (aside from 95% confidence interval) are provided to define each Phase for this population specifically, meaning that the method relies exclusively on the morphological descriptions that accompany each new Phase to estimate age. Although the provision of photographs and descriptions helps to establish the pattern of morphological change that occurs in this population, the lack of statistics (e.g. mean age and range per phase, standard deviation, standard error) significantly limits the accuracy with which an estimate can be made since error margins are not known or reported.

3.4.4. Salem et al (2014)

This study assessed the applicability of the Isçan Phase aging method in a Tunisian population. A total of 108 sternal ribs were collected from documented Tunisian males between 18 and 83 years of age at autopsy. Two judges assigned each rib to a given Phase based on photographic plates and descriptions provided in the original study (Isçan et al 1984b).

Intra-observer accordance (evaluated using the Kappa statistic) was reported as substantial at 0.73 and 0.71 for each operator (Landis & Koch 1977). Inter-observer accordance was substantial ($K = 0.747$) (Landis & Koch 1977), with both operators estimating the same Phase 78.7% of the time. Perfect agreement between the chronological age of the individual and the estimated Isçan Phase occurred predominately for Phase 5 and below for both observers. Overall, the Phases devised by Isçan et al were found to underestimate the age of Tunisian specimens for both observers. The 8 Phases (excluding Phase 0) in the original study were pooled into 5 Phases in the current study, given that the sample consisted mainly of individuals under the age of 56 years. The method was found to explain approximately 56.5% of variation in the appearance of features due to increasing age. The authors concluded that the Isçan et al standard cannot be applied to a Tunisian male population to estimate age (particularly in individuals over the age of 56), despite acceptable levels of inter- and intra-observer error.

The revised method presented by Salem et al (2014) cannot be considered useful for forensic purposes where a distinction between juvenile and adult remains is required, as individuals up to 30 years of age are grouped together in a single Phase, and the study failed to sample individuals in late adolescence and early adulthood to establish the age of transition from Phase 0 to 1.

3.5. Applicability of the method to other ribs

The exclusive use of the fourth right rib for age estimation has precluded the examination of the utility of other ribs in this process. Testing the applicability of the original method in other ribs increases its scope and provides a means of quantifying morphological changes in a greater range of skeletal elements. This is useful in both forensic and archaeological contexts, where the fourth right rib may be missing (or damaged), as it is a relatively fragile feature (Isçan et al 1984b). Alternate ribs may be required as a supplementary source of data, as long bony projections that are prone to damage can be diagnostic of one Phase over another (Loth et al 1994). The use of a more holistic method also overcomes problems associated with the isolation and positioning of the right fourth rib, as it is more difficult to discern between, and identify ribs other than the first, due to natural variation (Loth et al 1994).

3.5.1. Loth, Isçan & Scheuerman (1994)

This study aimed to determine the applicability of standards for Phase age estimation of the right fourth rib in the adjacent ribs three and five, and to quantify any Phase differences that may occur across ribs. The sternal rib ends of right ribs three, four and five (see Figure 3.7) were removed from 135 individuals at autopsy (102 males and 33 females of Caucasian ancestry, ranging from 3 to 99 years of age). Age was estimated using sex and ancestry specific standards developed by Isçan et al (1984b, 1985).

Ribs three and five were assessed to within 1 Phase of rib four 98% of the time, and all three ribs were judged as belonging to the same Phase 79% of the time across male and female specimens. Ribs three and five were judged as belonging to the same Phase as the fourth rib in 90% and 84% of individuals respectively. Rib three most closely resembled the fourth rib in its morphology and pattern of age related metamorphosis, although it tended to be under-aged when deviations were recorded. Intercostal variation was found to decrease with advancing age, with the greatest amount of variability observed in individuals under 30 years of age. Variation was observed to decrease significantly following the age of 40, and was reported to “completely disappear” (1994:141) in both sexes following the age of 80. No precision tests to quantify intra- and inter-observer error were performed. Given those results,

the authors concluded that standards developed for the estimation of age from the fourth right rib can be used to reliably estimate age in the right third and fifth rib.

Figure 3.7. Ribs 3, 4 and 5 (from left to right) from a 20-year-old male. All ribs were identified as belonging to the Phase 2 as defined by a shallow pit, minimal billowing, and rounded edges with scalloped edges (adapted from Loth et al 1994).

The results of this study can only be generalised to Caucasian American individuals (although with caution given potential temporal differences with contemporary populations) since these results are derived from a Caucasian American population. Phase similarities between the right ribs were not examined in other populations, meaning that it cannot be assumed that these similarities will necessarily hold true. The sex-bias in favour of male individuals limits the conclusions that can be drawn from female ribs. A lack of error estimates necessitates caution when applying standards from the fourth right rib to any other rib in the complete adult series.

3.5.2. Yoder, Ubelaker & Powell (2001)

This study tested the applicability of the fourth sternal rib aging technique to ribs 2 through 9 on both sides of the body. A total of 231 male and female ribs from The Terry Collection, The Maxwell Museum's Documented Collection, and the William Bass Donated Skeletal Collection were used in this study. Individuals were of both Caucasian American and African American ancestry, and ranged between 10 and 70+ years of

age. Rib specimens were analysed using the appropriate sex specific method devised by Isçan et al (1984b, 1985). Wilcoxin signed rank tests were performed to determine whether significant differences occurred between observed Phase scores based on the fourth rib, and those for the other ribs examined. Scores obtained from the ribs on the right side of the body were compared to those on the left (bilateral effects), and the fourth right rib specifically was tested against right ribs 2, 3, 5 through 9, left ribs 2 and 3, and the average score of a given individual's entire rib series (excluding rib 9). Differences based on sex and ancestry were evaluated for any significant difference between the right fourth rib and other ribs on the right side, and between right rib 4 and left ribs 2 and 3.

The authors reported no significant intra-observer error in fourth rib scores obtained from either the right or left rib series. Scores obtained from ribs other than the fourth right rib typically differed by one Phase. No significant differences between ribs 4 to 9 were observed for the right versus the left series. Scores from right ribs 3 through 9, and left rib 2, did not differ from the fourth right rib. Further, the average of a given individual's Phase score was not significantly different in either left or right rib series. A significant overall difference in Phase assignment according to right fourth rib standards was noted for the second right rib and the third left rib; these ribs were typically assigned to older Phases compared to their right series counterparts. The fifth right rib was the least different from the fourth right rib for females (different Phase was assigned 37.6% of the time), and the same was found for the sixth right rib in males (different Phase was assigned 36.1% of the time). When separated by ancestry, the second right rib and third left rib differed significantly from the fourth right rib for Caucasian and African American individuals respectively. Given results with mixed statistical significance, the authors recommend caution when estimating age applying the Isçan et al method (1984b, 1985) to ribs other than the fourth.

3.6. Contemporary challenges and revisions

Contemporaries of Isçan et al (1984a, 1985) have challenged the fundamental methodology and sampling procedures of the original method, claiming that it has been widely implemented without being challenged, citing bias, poor reproducibility of results and transposition of the method to other populations, small sample size,

moderate to high levels of inter- and intra-observer error, large age ranges and age mimicry as limitations (Fantón et al 2010; Hartnett et al 2010). Given the continual use of the fourth right sternal rib in contemporary age estimation (Garvin & Passalacqua 2012), the completion of independent validation studies is important to establishing the overall accuracy, transparency, reproducibility and validity of the original method in modern populations. The following section considers a selection of those validation studies.

3.6.1 Loth (1995)

This study aimed to determine if rib specimens from the Spitalfields cemetery population (archaeological sample of modern humans dating from the 16th to the 18th century) follow a similar pattern of morphological change as those that inform the standard for age estimation as devised by İşcan et al (1984b, 1985). A secondary aim was to determine the accuracy with which modern standards can be applied to an archaeological population. The examination of past archaeological populations is important in reconstructing the aging process of these populations, and to determine whether patterns of senescence deviate across temporally dissimilar populations. The age of 74 right fourth rib specimens (36 males and 38 females, ranging from 18 to 89 years of age) was estimated using the appropriate sex specific standard (İşcan et al 1984b, 1985).

The İşcan et al method was more accurate in estimating the age of male individuals (reported inaccuracy of 5.4 years, age underestimated by 2.9 years) and involved less inaccuracy when compared to age estimates made using the pubic symphysis and auricular surface (6.5 and 7.3 years respectively) following Lovejoy et al (1985b). Levels of bias were, however, comparatively higher than those reported by Lovejoy et al (age was overestimated by 0.4 years using the pubic symphysis and underestimated by 0.5 years using the auricular surface). Intercostal variation between ribs was minimal, where ribs three and five were used to estimate age to within one Phase of the fourth rib in 97% of cases. The author emphasises the importance of considering the 'feel' of bones in relation to age estimation, where the texture, density and firmness of a given specimen is significant to assigning it to a younger age Phase when the bone exhibits a

morphology macroscopically comparable to that of older specimens. However, it is also stated that this method should not be applied to archaeological remains, which is the nature of skeletons contained within the Spitalfields reference collection. It may be difficult to generalise these results to younger individuals, as individuals 60 years of age and older are over represented in this study (make up approximately 59% of the sample).

3.6.2 Hartnett (2010)

This study evaluated the Isçan et al method in a large, modern sample of individuals in order to introduce revisions and increase the accuracy of the method given previous criticisms concerning sample composition and size, and high levels of inter- and intra-observer error. A total of 630 fourth sternal rib ends obtained at autopsy (419 males and 211 females, ranging from 18 to 99 years of age) were used in this study. Age was estimated using written Phase descriptions (Isçan et al 1984b, 1985) and casts, where pit depth, the composition of rim edges and the overall quality of bone were considered to be the most discriminatory features.

Inter-observer error ranged between $r = 0.64$ and $r = 0.81$, and intra-observer error was reported as $r = 0.92$ for all observers on combined male and female scoring. Chronological age and estimated Phase scores were moderately correlated (between $r = 0.66$ and 0.72) across observers. Regression of the numerical age of individuals on established Isçan et al age Phases yielded a statistically significant result, with strong r values (0.875 for males and 0.924 for females). Standard deviations and age ranges derived from the autopsy sample were generally lower than those of the Isçan et al sample. Alterations to original bone morphology descriptions included avoidance of terms such as “becoming” and “lighter than,” and an increased emphasis on bone quality. The author concluded that the Isçan et al method is not sufficiently accurate for the estimation of age, given moderate correlation rates between the actual and observed ages of ribs.

3.6.3 Merritt (2014)

This study compared the reliability and accuracy of the original Iscan et al method to revised rib Phase aging standards published by Hartnett (2010) in a modern sample of 313 individuals taken from the William Bass skeletal collection (predominantly Caucasian sample with limited individuals of African American, Asian and Hispanic descent). Hartnett's revisions to the original Iscan et al method include changes to age ranges and mean age per Phase, modified descriptions of rib morphology (avoiding subjective descriptions such as 'becoming') and increased emphasis on the quality of bone, as this was found to improve Phase estimation. The mean age at death for each Phase was compared to actual age at death using the fourth right rib, or the third or fifth rib if it was found to be damaged or unavailable.

The level of intra-observer error for both the Hartnett and Iscan methods was near perfect at $K = 0.87$ (Landis & Koch 1977). The Iscan method estimated individuals to the correct Phase at a rate of 57.5%, compared to 29.7% using the Hartnett method. When examined according to sex, significantly more male and female individuals were assigned to the correct age Phase using the Iscan method (rate of 61.8% and 46.6% respectively) when compared to the Hartnett standard (rate of 31.6% of males and 25% of females). The same was observed according to ancestry, although this result was only significant for individuals of European ancestry (rate of 57.5% compared to 31.2% using the Hartnett standards).

Finally, the Iscan method was more accurate in assigning the correct age Phase in all age categories, except for those individuals between 80 and 89 years of age. No significant differences between methods was found in terms of inaccuracy (absolute error of age estimate without factoring in over or under estimation), although bias (mean over or under estimation) was found to differ significantly (-0.14 years using the Hartnett standard, compared to -4.72 years using the Iscan method). The Iscan method was superior when estimating the age of males (inaccuracy of 9.8 years with a bias of -1.6 years), while the Hartnett standard was superior for female individuals (inaccuracy of 14.63 years, with a bias of -8.97 years). The Iscan standard was more accurate for the estimation of age of African American, Asian and Hispanic individuals, while the Hartnett standard was more accurate for individuals of European ancestry.

While the Isçan et al standard was found to assign more individuals to the correct age Phase, both methods were found to perform poorly overall.

The main source of error associated with this study is the use of the third or fifth rib in place of the fourth. Previous studies have tentatively established that the right third and fifth ribs are potentially useful for the estimation of age in place of the fourth rib, although those studies are not without their limitations (see section 3.5.1 and 3.5.2) and have been conducted using a predominantly Caucasian population. Given the differences that occur in the aging process between Caucasian individuals and those of non-Caucasian descent, it cannot be assumed that alternate ribs in these ethnically disparate populations undergo morphological change at the same rate as the fourth rib within Caucasian populations. Further, there is comparatively limited data for individuals of African American, Asian and Hispanic ancestry (21 and 7 individuals respectively) within this study, and it is therefore difficult to make quantifiable conclusions concerning the rib traits of these ethnic groups. Rates of correct Phase assignment using this population and method were higher compared to those achieved by Hartnett (2010), although at 57.5% this rate is still relatively low.

3.6.4 Matrilie et al (2007)

The main aim of this study was to evaluate the accuracy of single versus multifactorial age estimation methods using a variety of morphoscopic techniques (based on the analysis of the pubic symphysis, auricular surface, sternal rib ends and monoradicular teeth). The accuracy of each individual method was evaluated using measures of inaccuracy and bias based on median age, and the overall accuracy of the use of all combined methods was determined using Principal Components Analysis (PCA). A total of 218 skeletons of Caucasian (55 males and 43 females between 25 and 85 years of age) and African American ancestry (60 males and 60 females between 26 and 91 years of age) taken from the Terry Collection were used in this study. Sex specific methods for the evaluation of the fourth right rib were employed (Isçan et al 1984b, 1985), and the third or fifth right rib was used if the fourth rib was unavailable.

The Isçan et al Phase aging technique was the most accurate method in estimating the age of elderly individuals (60+ years) across all specimens examined, particularly those

of Caucasian ancestry (median inaccuracy of 10.3, with a bias of ± 10.1 years) and male sex in the pooled ancestry sample (median inaccuracy of 8 compared to 9.5 for females across all ages). Overall, median inaccuracy scores for the Isçan method were lower than those found using PCA scores in the 60+ years age group. This is particularly significant as the use of multiple indicators of age is considered to be preferable to that of a single indicator (Franklin 2010). However, PCA scores were associated with less bias when compared to Isçan age estimates, and were found to be the most accurate for combined age groups across samples.

The authors did not account for the potential effect that ancestry exerts on the accuracy of age estimation by using standards formulated for Caucasian individuals on the African American sample. It is stated that a previous test on the overall sample indicated no differences between these two groups, although no reference is made to the results specifically and no other information on the nature of this test is provided. Isçan et al (1987) found that African American ribs tend to appear younger than their Caucasian counterparts and exhibit an altered general pattern of morphological change (see above for details). These demonstrated differences call into question the conclusions made by the authors. Further, the third or fifth rib was used in place of the fourth rib when it was unavailable. As mentioned previously (see Merritt 2014), several studies have found that the pattern of morphological change in these ribs is similar to that undergone in the fourth rib (Loth et al 1994; Yoder et al 2001) although data on African Americans is comparatively lacking, and significant differences in rib morphology between African American and Caucasian populations have been found (see Yoder et al 2001). Although the Isçan method may have been found to be most accurate for estimating the age of individuals over 60 years, it is still associated with high levels of inaccuracy and bias.

3.7 The sternal rib and virtual anthropology

The use of virtual data largely overcomes limitations associated with the direct observation of isolated dry bone specimens, such as taphonomic damage and problems related to identification and siding of the fourth right rib (Yoder 2001). Virtual data is non-invasive, can be readily accessed for the reproduction and validation of methods and results, and provides a means of developing contemporary,

population specific reference standards (Grabherr et al 2009; Brough et al 2012). The main disadvantage associated with the use of virtual data is the inability to palpate and kinaesthetically assess bone specimens (Hartnett 2010), which has been identified as an important means of distinguishing between Phases (Loth 1995). However, virtual repositories have been shown to be viable alternative sources of biological data for the purposes of age estimation using a variety of skeletal elements (Barrier et al 2009; Kellinghaus et al 2010; Wink 2014). The following study represents one of the only other known attempts to evaluate the efficacy of the Iscan method using a virtual set of skeletal remains.

3.7.1. Dedouit et al (2008)

This study aimed to test the applicability of the Iscan Phase aging method to two and three dimensional multi-slice computed tomographic reconstructions of the fourth right rib, and to then compare estimates to those derived from dry bone specimens to determine the inter-method accuracy of the technique (see Figure 3.8). A total of 36 fourth right rib specimens obtained from French individuals of known sex and age at autopsy were used in this study. The age of specimens was estimated using original Phase descriptions and photographs devised by Iscan et al (1984b, 1985) by three different observers with varying experience with the method. Morphological features considered to be diagnostic of age included wall thickness and the texture (porosity) of specimens, the appearance of the articular surface, pit, rib rim and edges and any bone projections.

The authors found that the accuracy of the Iscan et al standard did not differ based on choice of method of analysis (traditional versus virtual). The actual age of individuals was determined in 58.3% and 63.9% of cases based on the assessment of dry bone specimens and observation of MSCT data respectively. Intra-observer concordance associated with dry bone analysis was quantified with a gamma coefficient of 0.87, and 0.86 for MSCT reconstructions. Inter-observer concordance ranged from 0.73 to 0.91 on dry bones, and 0.82 to 0.88 for MSCT reconstructions. The inter-method error ranged between 0.55 and 0.71 across observers; approximately 80% of the estimates performed by the observer with experience in forensic anthropology on dry bone specimens and MSCT reconstructions agreed perfectly or were within one Phase of

each other. Agreement between Phase estimations generated by each respective method was found to be significant. The authors concluded that the transposition of traditional methods to a virtual platform is possible owing to the result that no statistically significant differences between the two methods were observed.

The main limitation of this study is the small sample evaluated. This is particularly significant as this study represents the first formal attempt to apply the Isçan standards to a virtual reconstruction of the fourth right rib. The small sample has resulted in the under representation (or complete lack) of individuals within the ranges of 10 to 19, 60 to 69, 70 to 79 and 80 to 99 years of age. Female individuals are not represented within the 10 to 19 years of age range, and the same goes for male individuals within the 80 to 99 age range. This lack of specimens calls into question the overall conclusions that are made about the applicability of the Isçan method to multi-slice rib reconstructions as they don't apply to individuals within these age groups, and thus cannot be used to validate the applicability of the method across the entire age range of Phases.

Figure 3.8. Dry bone specimens (top) compared to their corresponding MSCT reconstructions (middle and bottom). Plate A; 16-year-old male assigned to Isçan Phase 1. Plate H; 86-year-old male assigned to Isçan Phase 8 (adapted from Dedouit et al 2008).

3.8. Quantitative analysis

Recent revisions of the original Phase aging method have attempted to numerically quantify morphological changes that occur in components of the right fourth sternal rib to remedy perceived limitations associated with morphoscopic analysis (Hefner & Stephen 2012). Traditional morphoscopic techniques do not readily lend themselves to rigorous statistical analysis from which quantifiable conclusions may be drawn, given that they generate qualitative rather than quantitative data (Hefner and Stephen 2012). The attribution of a numerical score, rather than a description of the appearance of a given skeletal feature, permits the development of a statistical model for the estimation of age (Verzeletti et al 2010). The use of geometric morphometric techniques as an alternative method for the quantification of shape variables has also provided a means of statistically evaluating morphological differences between individuals. This technique employs statistical analysis of homologous three-dimensional landmark coordinates, allowing differences in shape between specimens to be evaluated irrespective of differences in size (Mitteroecker & Gunz 2009). A geometric morphometric approach towards age estimation relies on the quantification of shape differences that occur due to age related metamorphosis. This approach has generally been shown to be an effective method in developing standards for biological analysis in forensic anthropology (Franklin et al 2005, 2007). The following studies represent initial attempts to construct a viable numerical method that facilitates the statistical analysis of morphological changes within the rib.

3.8.1. Verzeletti et al (2010)

This study aimed to develop a numerical means of grading changes in the morphological composition of Components of the right fourth sternal rib as originally described by Isçan et al (1984a). A total of 49 sternal rib specimens collected from Caucasian Italian males at autopsy (ranging from 25 to 84 years of age) were evaluated. A regression equation for the estimation of age was developed based on the attribution of a numerical score to Components of the sternal rib end (articular surface, anterior and posterior rim walls and superior and inferior edges of the rib) (see Figure 3.9).

Figure 3.9. Schematic representation of morphological changes that occur within the articular surface. From left to right, representation of the deepening of the pit from a narrow V to wide set U. Taken from Verzeletti et al (2010).

The morphological appearance of the anterior and posterior rim walls was the most significant predictor of age ($r = 0.9399$), followed by the articular surface ($r = 0.9122$). The statistical significance of these correlations is not reported. The overall standard error for this method is ± 5.2 years, and ranged from ± 1.958 years for individuals between the ages of 20 to 29, to ± 6.278 years for individuals between 80 and 89 years of age. The results of this study can be called into question due to the small sample examined, the use of vague and inexact morphological descriptions and a lack of error quantification. Further, younger Phases are not examined and the authors fail to acknowledge the pattern of morphological change that characterises Phase 1 and 2. This may be problematic within a forensic context in circumstances where discrimination between juvenile and adult age status is required.

3.8.2. Verzeletti, Terlisio & De Ferrari (2013)

This study aimed to develop a method for the estimation of age based on the numerical quantification of the morphological appearance of the articular surface, anterior and posterior walls and margin of the fourth sternal rib end in a sample of Caucasian Italian female individuals (71 specimens ranging from 22 to 88 years of age) obtained at autopsy. The methodology of this study follows that described by Verzeletti et al (2010). A series of linear regression equations were established to estimate age based on scores obtained from the articular surface, antero/posterior walls, and rib margins (see Figure 3.10).

Articular surface (S)
S = 3		S = 6
S = 3.5		S = 7
S = 4		S = 8
S = 5		S = 9

Figure 3.10. Scores assigned to different articular surface morphologies. Red line indicates shape of the articular surface. Taken from Verzeletti et al (2013).

The shape of the articular surface was most strongly correlated with age ($r = 0.91$), followed by the appearance of the anterior and posterior walls of the rib ($r = 0.89$), and the rig margin ($r = 0.79$). A multiple regression formula combining these features was highly correlated with age ($r = 0.93$). No measure of significance for each regression and correlation are reported. These results support those found for males (Verzeletti et al 2010), suggesting that the articular surface is the most accurate predictor of age in this population. The efficacy of this study is compromised by the small sample, over representation of individuals over the age of 51 years, and the lack of quantification of inter-and intra-observer error.

3.8.3. Macaluso & Lucena (2012)

This study aimed to apply the method devised by Verzeletti et al (2010, 2013) to an autopsy sample of individuals from Southern Spain (58 males and 36 females; 18 to 89 years of age) to determine the accuracy of the novel Components method in this population, and to develop regression formulae specific to a Spanish population (if required). Substantial intra-observer agreement was recorded for the articular surface ($K = 0.70$), anterior and posterior rib wall ($K = 0.63$) and superior and inferior rib edge scores ($K = 0.72$) (Landis & Koch 1977). Numerical scores used to quantitatively describe the morphology of the articular surface and anterior and posterior rib wall configurations were found to significantly correlate with age across individuals ($r = 0.897$ and 0.837 for the male sample respectively; and $r = 0.783$ and 0.88 for the female sample respectively). Correlation coefficients for superior and inferior rib edge morphology were only weak to moderate ($r = 0.593$ for males and $r = 0.489$ for females), and were thus not considered to contribute to the final age estimate (Landis & Koch 1977).

The original Verzeletti method was shown to slightly overestimate the age of males (+0.78 years) and underestimate the age of females (-2.85 years). Overall, the method was associated with a combined inaccuracy of 6.53 years, and underestimated age by 0.61 years. The original regression formula was most accurate for individuals between the age of 18 and 29 years of age (lowest absolute error of 2.37 years in the pooled sample), and conversely, was the least accurate in individuals between the age of 80 and 89 years, where the age of specimens tended to be underestimated. Two regression equations specific to a Spanish population (where sex was included and excluded as an independent variable) yielded comparable results to that reported by Verzeletti et al (2010) (absolute error of 6.53 and 6.57 years respectively), although no significant age bias was found. The use of a sex specific equation did not increase the accuracy of age estimates when compared to the non-specific equation.

3.8.4. Nikita (2013)

The primary aim of this study was to statistically quantify differences in the morphology of sternal ribs three through ten (bilateral) to determine whether significant differences in shape occurred between individuals of various ages based on geometric morphometric digitising techniques. A secondary aim was to determine whether sternal rib shape differences were significant enough to impede the application of standards developed for the right fourth sternal rib to other ribs. A total of 73 specimens from documented female individuals belonging to the Spitalfields (53 specimens) and Saint Bride's (20 specimens) historical skeletal collections were analysed. Data was evaluated using the Phase standards developed by Isçan et al (1985) and pit depth as used in the original Components study (Isçan et al 1984a). A total of 11 Type III landmarks (Bookstein 1991) devised by the author were digitised on the relevant rib specimens (see Figure 3.11).

Ribs three and four on both sides exhibited no statistically significant morphological differences, although the fourth rib was found to vary significantly in its morphology compared to ribs five through ten. Cross validated discriminant analysis results were poor (ranging from 16.7% to 37.2% correctly classified on the right side, and 11.9% to 40% for the left side of the body based on Procrustes residual scores), suggesting no significant difference in the morphological shape of rib ends across individuals of different ages. A correlation between actual and the predicted age of a given individual was found, although the extent of the scatter of values precludes the use of this method for the estimation of age in contemporary individuals. Given the statistical benefits afforded by the application of geometric morphometric techniques, further research using a larger sample of contemporary individuals is required.

The conclusions reached by Nikita (2013) are somewhat counter intuitive given that the rib end undergoes significant morphological remodelling and change in its overall shape from adolescence to old age (refer to Figure 2.13). These unexpected results may be due to several factors, including sample size and composition and choice of landmarks. The conclusion that the rib does not undergo significant

Figure 3.11. Landmarks used to digitise the morphology of each rib. The landmarks are defined as follows; 1. The uppermost point on the rim; 2. The lowermost point on the rim; 3. The most anterior point on the rim; 4. The most posterior point on the rim; 5. The point at the projection of the middle of the line connecting the uppermost and the most anterior points on the rim; 6. The point at the projection of the middle of the line connecting the lowermost and most anterior points on the rim; 7. The point at the projection of the middle of the line connecting the uppermost and most posterior points on the rim; 8. The point at the projection of the middle of the line connecting the lowermost and the most posterior points of the rim; 9. The deepest point in the pit along the line connecting the most anterior and the most posterior points on the rim; 10. The point at the projection of the middle of the line connecting the deepest point in the pit and the most anterior point on the rim; 11. The point at the projection of the middle of the line connecting the deepest point in the pit and the most posterior point on the rim. Taken from Nikita (2013).

changes in shape as a function of age applies exclusively to the sample population, since the specimens used in this study derive from historical individuals that are not necessarily representative of modern populations. The Spitalfields collection is comprised of approximately 1000 individuals dating from 1729 to 1852, of which 400 have been formally identified (Molleson et al 1993). These individuals are predominantly Huguenot in origin (French Protestant refugees fleeing France due to religious persecution), although the sample includes individuals of English and Swiss ancestry (Molleson et al 1993). Although such collections provide valuable data on the growth, senescence and health and disease profiles of past populations that can be

likened to contemporary populations, their utility is impeded primarily by secular and temporal differences with their contemporary counterparts.

The lack of significant shape differences between individuals of different ages could in part be explained by the nature of the landmarks that were selected. A landmark is defined as a discrete point that is considered to be biologically homologous (that is, occurring in specific locations within and between specimens) amongst all individuals that form a particular population or data set (Bookstein 1991; Mitteroecker & Gunz 2009). Bookstein (1991) categorises landmarks into three different types; extremal points or Type III landmarks that occur 'farthest' from other points are considered to be the least accurate as their biological significance is unclear (Bookstein 1991). Given the curved nature of the sternal rib end, the use of semi-landmarks in a study of this nature may have been more appropriate. The use of semi-landmarks on curved surfaces overcomes limitations associated with the use of traditional landmarks, in that homology is geometrically established through the spacing and sliding of landmarks, meaning that shape data can be captured more accurately (Gunz & Mitteroecker 2013). It should be noted that the placing of semi-landmarks must be motivated by the biological proposition being tested, and should fulfil the requirement of homology (Gunz & Mitteroecker 2013).

The location of landmark 9 (the deepest point in the pit along the line connecting the most anterior and the most posterior points on the rim) is akin to pit depth described by Isçan et al (1984a) as part of the Components method (the maximum dimension of the cavity that forms in the medial articular surface of the rib with age defined as the distance between the bottom of the pit and the greatest height of an adjacent rib wall measured by a set of callipers); the use of this landmark may not be appropriate given that Isçan et al (1984a) found that pit depth did not provide an accurate indicator of age (partial eta of 0.41), particularly after 50 years of age. This feature was not utilised as part of the subsequent Phase based method (Isçan et al 1984b). Further, in a critical observation study of the fourth sternal rib end, Fanton et al (2010) found that the reproducibility of pit depth measurements was poor across observers, citing the presence of multiple potential

measurement points, bone projections and windows, and irregularity of the pit and rim as reasons for this result. Capturing the location of this landmark with a digitiser using a dry bone specimen could also prove difficult, particularly with older specimens that may be fragile or significantly deteriorated.

3.9. The contribution of the present research thesis

The generation and use of population specific reference standards is central to the creation of the most accurate biological profile possible in forensic anthropology. Given the current (relative) lack of standards for an Australian population in general, and a Western Australian population specifically, the ultimate contribution of the present thesis is the creation of reference standards for the estimation of age based on sternal rib morphology specific to a Western Australian population. The ribs represent a novel skeletal site for the estimation of age in this population, thereby increasing the amount of available data for the compilation of the biological profile. In addition, this study further contributes to the existing body of literature that is concerned with the transposition of traditional analytical techniques to a virtual platform, and the validation of these novel approaches

Chapter Four: Materials and methods

4.1. Introduction

The overall aim of the present thesis is the generation of standards for the estimation of age specific to a Western Australian population based on the sternal rib ends. The current chapter accordingly describes the materials, methods and statistical analyses utilised in order to fulfil this objective. Statistical methods presented include precision tests to assess the level of intra- and inter-observer error involved in the analysis of computed tomography (CT) scans, tests of the normality of data, and a selection of parametric tests for the assessment of mean age and standard error differences for the construction of population specific standards. Transition analysis was also performed to provide a more comprehensive understanding of the precise rate of sternal rib metamorphosis relative to age.

4.2. Materials

The present study is based on the retrospective analysis of thoracic multi slice computed tomography (MSCT) scans collected from a Picture Archive Communication System (PACS) database. The PACS database represents a digital repository of clinical images generated using multiple imaging modalities of patients presenting to hospitals within the Perth metropolitan area. The necessary scans were acquired by Dr Rob Hart (UWA Clinical Associate Professor, Department of Radiology, Royal Perth Hospital) and were anonymised prior to receipt by the author (devoid of all personal information other than the date of the scan, sex and date of birth of the individual; information regarding ancestry is not recorded as it is not considered to be medically relevant). Scans were obtained using a Philips Brilliance 128 slice CT scanner (acquisition of 120kVp, mAs 113, with a pitch of 1.172 and rotation time of 0.4 seconds) with high resolution. Slice images were subsequently reconstructed at a thickness of 0.8 mm (at an increment of 0.4 mm) with an image matrix of 512 x 512 pixels (window centre of 40 and width of 350).

4.2.1. Sample demographics

The assembled sample represents scans of individuals between 10 and 80 years of age presenting at public hospitals with clinical complaints relating to abdominal and thoracic viscera. The contemporary Western Australian population is diverse in its cultural and ancestral composition, with 17.5% of the Greater Perth population and 15.9% of the state population overall born overseas (see Tables 4.1 and 4.2; this data represents the most common ancestries reported in the 2016 census data; Australian Bureau of Statistics 2017). Indigenous individuals and Torres Strait Islander peoples constitute 1.6% and 3.1% of the Greater Perth and Western Australian population respectively. Despite being ancestrally diverse (see Tables 4.3 and 4.4), the majority of Western Australian individuals are Caucasian with a European lineage.

Table 4.1. Proportion of the Greater Perth (GCCSA) population born overseas (based on 2016 census data).

Geographic region	Percentage of the GCCSA born overseas
England	8.6
New Zealand	3.2
India	2.4
South Africa	1.8
Malaysia	1.5

Table 4.2. Proportion of the overall Western Australian population to be born overseas (based on 2016 census data).

Geographic region	Percentage of the WA pop. born overseas
England	7.8
New Zealand	3.2
India	2.0
South Africa	1.7
Malaysia	1.2

Table 4.3. Self-perceived ancestry of individuals residing in the Greater Perth (GCCSA) region (based on 2016 census data).

Ancestry	Percentage
English	27.5
Australian	20.9
Irish	6.8
Scottish	6.4
Italian	4.0

Table 4.4. Self-perceived ancestry of all Western Australian individuals (based on 2016 census data).

Ancestry	Percentage
English	27.9
Australian	22.8
Irish	6.7
Scottish	6.4
Italian	3.7

4.2.2. Inclusion and exclusion criteria

A total of 529 scans were originally acquired for this project. The integrity of each scan (slice thickness, resolution, visualisation of sternal rib ends) was assessed prior to any formal analysis and statistical testing. Scans were excluded on the basis of poor visualisation of skeletal structures (including but not limited to scans with a slice thickness over 1mm with poor resolution); evidence of thoracic skeletal trauma, pathology, malformation or abnormal growth; obscuration of skeletal features by medical devices that could not be removed without also removing essential skeletal features; and complete ossification of ribs to the sternum. Scans were excluded according to the aforementioned criteria over two rounds. The first round occurred prior to initial data analysis, and the second following initial examination of the distribution of data, to remove individuals that would potentially adversely impact

results due to issues such as subpar resolution. The final sample used in the statistical analysis consisted of 335 individuals (179 males and 156 females), from 10 to 80 years of age (refer to Figure 4.1).

Figure 4.1. Age and sex distribution of individuals within the study sample.

4.2.3. Human ethics approval

Human ethics approval was required prior to the receipt of scans and data collection; this was granted on the 2nd of May 2016 by the University of Western Australia's Human Ethics Research Committee (RA/4/1/4362; see Appendix 1). This project has been annexed to Associate Professor Daniel Franklin's HREC research scheme entitled 'Novel approaches to the forensic identification of human remains: bone morphometrics.'

4.3. Methods

4.3.1. Visualisation protocol

Digital Imaging and Communications in Medicine (DICOM) files representing MSCT thoracic or abdominal scans were visualised and analysed in a Macintosh platform (OS X Yosemite version 10.10.3) running the 64-bit version of the *OsiriX* Imaging software. *OsiriX* is an open source, digital DICOM viewer designed to facilitate the visualisation and examination of multidimensional images generated by various advanced medical imaging modalities, such as positron emission tomography and CT (Ratib & Rosset 2006). The 3D viewing mode of the program was used to reconstruct CT slice images using volume rendering multi-planar reconstruction, surface rendering and maximum intensity projection protocols (Ratib & Rosset 2006), resulting in a 3D volume rendering of the fleshed or skeletonised thorax and/or abdomen of each individual (see Figure 4.2; left and right images respectively).

A combination of different digital manipulations was performed in order to achieve the clearest visualisation of the rib end possible. The same suite of visualisation methods and protocols were used to analyse all scans, although to varying degrees according to the individual properties of each scan (e.g. some scans required more contrast adjustment or magnification to clearly make out rib features). The contrast adjustment function was utilised in all cases to enhance the appearance of rim irregularities, pit depth and pit features, such as bone projections and the overall quality of bone. The 2D orthogonal MPR view was used to discern the shape of the pit when it was difficult to visualise this feature using the 3D volume rendering alone (see Figure 4.3). The low contrast, bone glossy preset was considered most appropriate for the visualisation of

skeletal structures in cases where these could not be visualised using the default 3D volume rendering setting alone (see Figure 4.2; right image). All features that were not of interest (e.g. extraneous medical devices, skeletal features other than that being examined) were removed using the scissor function. Each rib was examined in isolation from all other ribs and skeletal features using the setting that allowed examination at the finest level of detail.

Figure 4.2. Three dimensional virtual rendering of the fleshed (left) and skeletonised thorax (right) of a 17-year-old male used in the current study.

Figure 4.3. Screen shot of a 2D orthogonal MPR view of the right fourth rib (white structure in the centre of the image) of a female used in the current study. This view was utilised to determine pit depth and shape (denoted by arrow) when these features could not be clearly visualised using 3D rendering alone.

4.3.2. Morphoscopic sternal rib analysis

The age of each individual is estimated directly in CT scans following the visualisation of thoracic skeletal features. This was accomplished by virtually dissecting out all other skeletal features using the scissor tool, such that the medial surface of the rib of interest could be viewed in isolation (see Figure 4.4). Each rib was viewed from a number of different anatomical aspects using the box tool available in *OsiriX*, which permits 360-degree manipulation of a structure of interest. This method was chosen (as opposed to the analysis of a static image of the rib) as it most closely resembles the method of rib examination employed in assessing a physical, dry bone specimen (rib can be viewed from a variety of angles). An estimate of skeletal age was made by assessing the shape, form, texture and overall quality of bone observed in each scan, as well as the presence of certain characteristics that would suggest an estimate of one Phase over another (e.g. rib with a smooth, solid, flat medial articular surface with rounded edges is more likely to be estimated as Phase 0 over Phase 8). The appearance of Phase characteristics (pit shape and depth, rim configuration, presence of billowing or bony growths, porosity and texture of bone) evident in the scans was

compared with sex specific photographs, descriptions and reference casts (Isçan 1984b, 1985, 1993) detailing expected rib morphology (i.e. specific presentation of Phase characteristics) for each Phase (see Tables 4.5 and 4.6; Figures 4.5a-c and 4.6a-c). An estimate of age was produced when observed rib morphology was considered to correspond to that outlined for a particular Phase. Each rib in the series examined was analysed independently on separate occasions to prevent observer recall and bias.

Figure 4.4. Fourth right sternal rib of a female individual used in the current study viewed from anterior and medial anatomical aspects (same individual as pictured in Figure 4.3).

Table 4.5. Male sternal rib morphology descriptions for each Phase (adapted from Isçan et al 1984b). Plate numbers refer to those pictured in Figures 4.5a–4.5c.

Phase number	Phase description
Phase 0	The articular surface of the rib is flat with a rounded edge, a regular rim and no pit formation. The bone is smooth, firm and solid in appearance (plate 0a, b and c).
Phase 1	The articular surface is beginning to exhibit an amorphous indentation or pit, and may show evidence of billowing (plate 1b and 1c). The rim is still round and regular, although scalloping may occur (plate 1a). The bone itself is still smooth, firm and solid.
Phase 2	The pit has deepened and assumed a 'V' shape that is formed by the anterior and posterior rib walls. Rib walls are thick and smooth, with a scalloped or slightly wavy rim with rounded edges (plate 2a). The bone is solid and firm.
Phase 3	The pit has deepened and taken on a slight 'U' shape (plate 3b and 3c). Rib walls are relatively thick with rounded edges. Some scalloping may be present, but the rim is becoming more irregular in shape (plate 3a). The bone is still fairly thick and solid.
Phase 4	The articular pit is increasingly deep, and is a moderate to wide 'U' shape (plate 4b). The walls have rounded edges and are becoming increasingly thin (plate 4c). The rim is irregular and no longer exhibits a regular scallop pattern. The overall quality of bone is good, although a decrease in weight and firmness may be observed.

Phase number	Phase description
Phase 5	<p>Little change in pit depth, retaining a moderately wide 'U' shape. Progressive thinning of the rib walls can be observed, along with increasing irregularity of the rim and rib edges (plate 5a). Scalloping is absent and is replaced with irregular bony projections (plate 5c). Bone is in fairly good condition with some evidence of increased porosity (plate 5c).</p>
Phase 6	<p>The pit is noticeably deep and takes on a wide 'U' shape. Rib walls are thin with sharp edges; the rim has become irregular and exhibits long bony projections that are most pronounced at the superior and inferior rib borders (plate 6a, b,c). Bone is noticeably lighter in weight, and is thinner and more porous particularly within the pit.</p>
Phase 7	<p>The pit is deep with a wide to very wide 'U' shape. Rib walls are fragile and are associated with sharp, pointy and irregular bony projections (plate 7a). Bone is light weight and brittle with significant deterioration in porosity and quality (plate 7b, c).</p>
Phase 8	<p>The pit is very deep and has assumed a very wide 'U' shape (plate 8c). In some instances, the floor of the pit is absent or is filled with bony projections. Rib walls are extremely fragile, thin and brittle with sharp, high irregular edges and bony projections (plate 8a, b). The bone is very lightweight, brittle, thin and porous. 'Window' formations (gaps within the rib walls) can be observed in some cases.</p>

Figure 4.5a. Photographic plates that accompany Iscan standards for male individuals (1984b); Phase 0 to 2. See Table 4.5 for a description of associated morphology.

Figure 4.5b. Photographic plates that accompany Işan standards for male individuals (1984b); Phase 3 to 5. See Table 4.5 for a description of associated morphology.

Figure 4.5c. Photographic plates that accompany Ischan standards for male individuals (1984b); Phase 6 to 8. See Table 4.5 for a description of associated morphology.

Table 4.6. Female sternal rib morphology descriptions for each Phase (adapted from Isçan et al 1985). Plate numbers refer to those pictured in Figures 4.6a.-4.6c.

Phase number	Phase description
Phase 0	The articular surface of the rib is almost flat (no pit formation) with ridges or billowing present (plate 0c). The outer surface of the sternal extremity of the rib is bordered by an overlay of bone (plate 0a and b). The rim is regular in appearance with rounded edges. The bone itself is smooth, firm and very solid.
Phase 1	An amorphous indentation can be seen forming within the articular surface, and ridging or billowing may still be present (plate 1b, c). The rim is rounded and regular with some waviness in some instances (plate 1a). The bone is still smooth, firm and solid.
Phase 2	The pit has become deeper and has assumed a 'V' shape between the anterior and posterior rib walls (plate 2b, c). The pit may contain some evidence of bone ridging or billowing. The rim is wavy in appearance with some scalloping beginning to occur at the rounded edges (plate 2a). The bone itself remains firm and solid.
Phase 3	A slight increase in pit depth can be observed, and the structure overall assumes a wider 'V' shape that approaches a narrow 'U' shape as the rib walls become thinner (plate 3b, c). Rounded edges begin to exhibit a more regular and pronounced scalloping pattern (plate 3a). The anterior and/or posterior walls may begin to exhibit a central, semicircular arc of bone (plate 4c). The bone remains firm and solid.
Phase 4	There is a notable increase in the depth of the pit, which exhibits a wide 'V' or narrow 'U' shape with flared edges in some cases (plate 4b). Rib walls are thinner with rounded edges. Some evidence of scalloping is present along with the central arc; the scalloping pattern is less well defined and the edges appear to be worn down. There is a decrease in the density and firmness of bone and plaque like growths may appear within the pit (plate 4c).

Phase number	Phase description
Phase 5	<p>The pit remains the same depth as in Phase 4, and takes on a wider or flared 'V' or 'U' shape as the walls become thinner (plate 5b, c). Smooth, hard, plaque like deposits line the majority of the pit in most cases (plate 5c).</p> <p>Regular scalloping along the rib edge is absent, and the edge begins to become sharper in appearance. The rim is more irregular, with the central being the most prominent projection. The bone is noticeably lighter in weight, density and firmness, and is somewhat brittle in texture.</p>
Phase 6	<p>The pit has increased in depth and exhibits a widened 'V' or 'U' shaped appearance with pronounced flaring at the end (plate 6b, c). Plaque like pit deposits may occur but are rougher and more porous in appearance. Rib walls are quite thin with sharp edges and an irregular rim (plate 6b, c.). The central arc is becoming less obvious and in the majority of cases, contains sharp points that project from the rim (plate 6a). The bone itself is fairly thin and brittle with some deterioration.</p>
Phase 7	<p>The depth of the pit decreases slightly. Irregular bone growths are often observed extruding from the interior of the pit (plate 7b, c). The central arc is still present and is in most cases accompanied by pointed bony projections that occur along the superior and inferior rim borders (although can occur anywhere along the rim (plate 7a). Rib walls are very thin and exhibit an irregular shape with sharp edges. Bone is very light, thin, brittle and fragile with deterioration most noticeable within the pit.</p>
Phase 8	<p>The pit is 'U' shaped and is relatively shallow and badly deteriorated, completely eroded or filled with bony growths. The central arc is unrecognisable, and walls are extremely thin and fragile with a highly irregular rim and sharp edges with projections at the superior and inferior borders (plate 8a). Bone is extremely fragile, brittle and thin with window formation within walls occurring (plate 8c).</p>

Figure 4.6a. Photographic plates that accompany Ischan standards for female individuals (1985); Phase 0 to 2. See Table 4.6 for a description of associated morphology.

Figure 4.6b. Photographic plates that accompany Işcan standards for female individuals (1985); Phase 3 to 5. See Table 4.6 for a description of associated morphology.

Figure 4.6c. Photographic plates that accompany Ischan standards for female individuals (1985); Phase 6 to 8. See Table 4.6 for a description of associated morphology.

4.4. Statistical analyses

The following section outlines the statistical analyses performed. Intra- and inter-observer precision is quantified prior to subsequent parametric statistical tests that are used to evaluate the relationship between estimated and chronological age, and to compare means across ribs examined to evaluate differences in the pattern and rate of morphological change due to senescence. Subsequent polynomial regression and transition analysis statistics are used to formulate age estimation standards, and the standard error of the estimate (SEE) is used to assess the accuracy of the Iscan standard in a Western Australian population. The use of statistical methods presented in the following sections is governed by a number of assumptions that are specific to each individual method. Those assumptions are considered where necessary, but in general include (albeit not limited to), normality of data (scores should be normally distributed) equality of variances (homoscedasticity and sphericity), and independence of observations (Pallant 2011). Violations of those assumptions and the specific methods used thereafter are considered where relevant.

Normality of the data is assessed using the Kolmogorov-Smirnov statistic, where a significant result indicates that the data violates assumptions of normality (Pallant 2011). Appraisal of the normality of data is critically important as it is an assumption that is central to a wide variety of statistical methods of analysis, and can lead to inaccurate conclusions being made about data when the assumption of normality is violated without prior knowledge (Ghasemi & Zahediasl 2012). Sphericity or equal variance between population scores is assessed using Mauchly's test of sphericity and Levene's F test, where a non-significant result ($p > 0.05$) indicates equal population variance (Pallant 2011). Transition analysis is performed using the Nphases software available at <http://konig.la.utk.edu/~npahses2.htm>. All remaining analyses are performed using the *Statistical Package for the Social Sciences*, (IBM SPSS Statistics 23) and Microsoft Excel (Microsoft Excel for Mac, Version 15.24).

4.4.1. Intra- and inter-observer error

A precision test to quantify the level of intra-observer error related to age estimation in the right fourth sternal rib following the Iscan et al method was performed prior to primary data collection. Precision tests on ribs three and five were not performed due

to time constraints. The term ‘precision’ refers to the closeness of two or more repeated measurements of the same phenomenon or quantity, and is ultimately related to the overall reproducibility of a measurement (Harris & Smith 2009). Skeletal age of 40 randomly selected individuals (equally divided by sex and representing all Phase intervals) were estimated on two separate occasions by the author, with an intervening period of at least five days between re-assessment to minimise data recall.

Quantification of inter-observer error occurred following main data collection, and was completed using a randomly selected subset of 20 individuals from the original sample (similarly equally divided between males, females and Phase interval) by a PhD candidate familiar with the Iscan method. A total of two assessments made on separate occasions with at least five days between them were performed by this individual. The appropriate standards and prototypic ribs were used in all assessments (Iscan et al 1984b, 1985, 1993).

Cohen’s Kappa statistic was used to determine the level and significance of agreement between repeat Phase assessments for both observers. The Kappa statistic is used to quantify the magnitude of agreement between two independent observers or repeated measurements, and ranges from 0 (level of agreement equivalent to that that would occur by chance) to 1 (perfect agreement) (Viera & Garrett 2005; see Table 4.7). The higher the level or strength of agreement, the more precise the estimate. The calculation of this statistic is based on the difference between observed values (level of agreement that is present) and the level of agreement that is expected by chance (Viera & Garrett 2005); the Kappa statistic is defined by the following (Cohen 1960; Landis & Koch 1977):

$$\kappa = \frac{\text{Pr}(a) - \text{Pr}(e)}{1 - \text{Pr}(e)}$$

where $\text{Pr}(a)$ = observed agreement between raters or repeat ratings

$\text{Pr}(e)$ = expected level of agreement due to chance

Table 4.7. Kappa statistic ranges and associated strength of agreement benchmarks (adapted from Landis & Koch 1977).

Kappa Statistic	Strength of Agreement
< 0.00	Poor
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost Perfect

4.4.2. Descriptive statistics

Descriptive statistics are obtained prior to the analysis of relationships between estimated age (Phase) and the actual chronological age of individuals included in this study. Descriptive statistics are used to summarise the characteristics of a given sample and form the basis for conclusions that may be drawn about the overall population from which the study sample is derived (Sheats & Pankratz 2002). Calculated statistics mirror those obtained by Isçan et al (1984b, 1985) and include the mean age, range, standard deviation, standard error and 95 per cent confidence interval per Phase and the method overall.

4.4.3. Comparison of means

i) Analysis of variance (ANOVA)

An analysis of variance or ANOVA is a parametric statistical test used to compare the mean scores of at least three groups on a single or multiple independent variables, with each variable containing a number of levels (Dytham 2010; Pallant 2011). This test assumes that data are continuous, normally distributed and exhibit homogeneity of variance (Dytham 2010; Pallant 2011). A repeated measures design is used to compare mean scores in cases where three or more scores or observations are recorded for a single individual on three or more separate occasions (Pallant 2011). A one way (single independent variable) repeated measures ANOVA is performed to determine whether there is a significant difference in the mean age that is estimated between ribs three,

four and five (using pooled data and split according to sex) in a Western Australian population.

ii) Independent samples t -test

An independent samples or between subjects t -test is used to evaluate the difference between mean scores belonging to two populations, or two unrelated treatment conditions (Gravetter & Wallnau 2009). A between subjects design requires that observations or scores are independent from one another (not biased by other measurements or scores) and exhibit equality of variance, measured by Levene's F test (Pallant 2011). In the current study, an independent samples t -test is performed to determine if the mean estimated age for each rib differs according to the sex of the individual.

iii) Paired samples t -test

In contrast to an independent samples t -test, a paired or dependent samples t -test is used to determine whether the mean difference between two sets of observations (or data sets) relating to the same group of individuals across two different time points is different from 0 (no change in score or observation across time) (Townsend 2012; Wallenstein et al 1980). This test assumes that paired scores are normally distributed, independent from one another and exhibit homogeneity of variance (Pallant 2011). A series of paired sample t -tests are performed to determine whether the mean age estimated between rib pairs varied (i.e. rib three compared to rib four, rib three compared to rib five, and rib four compared with rib five). These tests were conducted using both the entire sample (pooled sex) and on data split according to sex.

4.4.4. Regression analyses

Correlation and regression analyses are mathematically related statistical techniques used to assess relationships or associations between variables, and predict values of one variable or measurement according to the other based on a presumed linear relationship between the two (Dytham 2010; Pallant 2011; Townsend 2012).

Regression differs from correlation in that one variable depends on and is predictive of the other, while correlation simply evaluates the strength of association between two variables (Dytham 2010; Pallant 2011). Both tests assume random data sampling,

independence of observations and normality of data (Townsend 2012). The standard error of the estimate (SEE) is used to measure the accuracy of predictions associated with a regression formula (distance between data points and the regression line), and is related to the strength of the association between x and y variables (Gravetter & Wallnau 2009).

i) Correlation

Parametric correlation testing requires the use of Pearson's product moment correlation coefficient (r), which is designed to measure the direction and strength of the linear relationship between two continuous variables (Pallant 2011; Townsend 2012). The r coefficient that is produced takes on any value between -1 and 1, indicating either a negative or positive association between variables, and the strength of the association; a value of 0 is indicative of no relationship, and a value of 1 suggests a perfect correlation, such that the value of one variable could be predicted perfectly by the value of the other (Pallant 2011). A bivariate Pearson correlation between estimated age at each rib pair (see above; paired samples t -test) is performed to determine the strength of the relationship of age related change across rib ends examined (whether age related morphological change is consistent across ribs). A second series of correlations between actual chronological age and that estimated for each rib is to be undertaken to determine the association between the Isçan method and actual age for individuals within a Western Australian population.

ii) Simple linear regression

Simple linear regression is used to examine the relationship between a single dependent or effect variable (y) and an independent or predictor variable (x), where a value of y can be predicted from any given value of x (Dytham 2010). This statistical procedure is closely related to Pearson's correlation, in that the method tests for a linear relationship (defined by a straight line) between variables (Townsend 2012). However, unlike Pearson's correlation, linear regression is used when the effect and predictor variables are considered to be dependent from each other so that the value of one variable can be predicted from the other (Townsend 2012). Linear regression analysis produces the following equation;

$$Y = bX + a$$

Where b = slope; a = y intercept

The slope of the resultant equation determines the amount of change in the y or dependent variable when the value of x or the independent variable is increased by 1, and the y intercept represents the value of the dependent variable when x equals 0 (Gravetter & Wallnau 2009). A simple linear regression is performed in the current study to determine if the actual or chronological age of individuals can be estimated using Phase scores determined by Isçan (1984b, 1985).

iii) Polynomial regression

Polynomial regression is a specialised type of linear regression in which the data does not conform to (or cannot be accurately predicted by) the use of a straight line of best fit, and instead requires the use of a curved line to better predict variable values (Dytham 2010). Non-linear or curvilinear relationships require the use of at least two predictor variables (x; independent variable) with a single dependent variable (y), where successive x power values within the model (see equation below) represent each respective predictor variable (Armitage et al. 2008);

$$E(y) = \alpha + \beta_1x + \beta_2x^2 + \dots + \beta_px^p$$

In the present study this type of regression is used to examine the relationship between actual age (y) and estimated age per rib (successive x values). The power of x (represented by p in the equation) is referred to as the degree of the polynomial and determines the overall shape of the model (e.g. quadratic, cubic, quartic) (Armitage et al. 2008).

iv) Multiple linear regression

Multiple linear regression is a statistical technique related to linear regression that utilises multiple independent variables (x) within a regression equation to improve the prediction accuracy of a single dependent variable (y) (Gravetter & Wallnau 2009; Townsend 2012). A multiple linear regression is performed in the present study to determine if the accuracy of chronological age estimation from observed Phase scores

can be improved with the use of multiple scores from different ribs. Phase scores from ribs three to five are introduced into the regression using enter and stepwise methods. The enter method inserts all independent variables into the equation simultaneously, so that they explain variance in the dependent variable as a cluster or group (Pallant 2011). In contrast, the stepwise regression method allows independent variables to be included or excluded from the equation in a particular order by the statistical analysis software, according to what combination generates the best fit model (model with the smallest residual values) (Pallant 2011; Townsend 2012). The use of a stepwise multiple regression model is considered to be advantageous as it identifies and includes variables that improve the overall accuracy of the regression (Dytham 2010), although it can be sensitive to outliers (Pallant 2011).

4.4.5. Western Australian standards

i) Transition analysis

Transition analysis is a relatively novel method of age estimation that attempts to overcome limitations such as age mimicry; the use of discrete age intervals and resulting large age ranges that are associated with traditional adult age estimation methods (Boldsen et al. 2002). This form of analysis provides an estimate of the average age at which a given individual is likely to transition from one age phase to another in a hierarchy of ordered, invariant senescent changes (Konigsberg et al 2008; Langley-Shirley & Jantz 2010). The use of transition analysis is favoured over other percentile methods that are sensitive to developmental outliers and small sample sizes (Shirley & Jantz 2011), and is applied in the present study as it provides an alternative method to the use of regression analysis for the estimation of age and modelling of thoracic senescence in a Western Australian population.

Transition analysis utilises an ordinal probit regression model to produce maximum likelihood estimates that represent the average age at which an individual is likely to transition from one age phase to the next (Boldsen et al. 2002; Shirley & Jantz 2011). This data can subsequently be transformed to generate transition curves, which graphically represent this process. Transition analysis is performed using pooled and sex-specific data for each rib to derive standards according to whether the sex of an

unknown individual is known or not. All analyses were performed using the multiple SD model available within the NPhases program.

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Chapter Five: Results

5.1 Introduction

The intended aims of the present study are as follow: i) to determine whether sternal ribs can be reliably assessed in clinical multi-slice computed tomography (MSCT) scans; ii) to determine if age related metamorphosis of the fourth right sternal rib follows the same pattern of progression in a Western Australian population compared to the American Caucasian population described by Isçan and; iii) to develop Western Australian reference standards for the estimation of age from the sternal rib ends as visualised in MSCT scans. Accordingly, the following chapter presents the results of the current study in the context of the aforementioned aims.

The results of intra- and inter-observer precision tests are first presented prior to consideration of the descriptive statistics for the ribs assessed. This is followed by a series of pooled sex age distribution plots for the purposes of assessing and comparing the rate of morphological change per Phase in the different ribs examined in the sample population. Inferential statistics for the comparison of means and assessment of the relationship between actual (or chronological age) and estimated age per rib are presented thereafter. The results of regression and transition analyses are finally provided to model predictive standards based on the analysis of right ribs three through five. This includes consideration of the overall accuracy of the Isçan method in the Western Australian population, which is evaluated using the standard error of the estimate statistic. The chapter concludes with a novel set of sex specific age estimation standards for a Western Australian population based on the fourth right sternal rib.

5.2. Intra- and inter-observer error

The following section outlines the results of precision tests used to assess intra- and inter-observer accordance in the estimation of Phase scores from scans of the fourth right rib end. Levels of intra- and inter-observer accordance were calculated using Cohen's Kappa statistic (Cohen 1960), based on a sample of 40 randomly selected scans (evenly distributed by sex and age) and a subsample of 20 scans (also evenly

distributed by sex and age) obtained from the original precision test sample, respectively. In each case, assessments following Işcan et al (1984b, 1985) were made on two separate occasions at least five days apart. Intra-observer concordance was ‘substantial’ (Landis & Koch 1977) with a Kappa value of 0.76 ($p < 0.001$). Inter-observer concordance was ‘almost perfect’ (Landis & Koch 1977) with an associated Kappa value of 0.825 ($p < 0.001$).

5.3. Tests of normality

The Komogorov-Smirnov test of normality is used to assess the distribution of Phase scores for each rib in the pooled and individual sex samples. This statistic was obtained prior to any descriptive or inferential statistics to determine the applicability of parametric statistics. The Komogorov-Smirnov test values were statistically significant across all ribs within the pooled and individual sex samples ($p > 0.001$; see Table 5.1). This indicates that the Phase values are not normally distributed and violate the assumption of normality. However, because the sample used in the current study is large (over 40 individuals), the violation of the assumption of normality does not preclude the application of, nor compromise interpretations from, parametric statistical analysis methods (Ghasemi & Zahediasl, 2012).

Table 5.1. Komogorov-Smirnov test for the distribution (normality) of Phase scores per rib.

Sex	Rib no.	N	KS statistic
Pooled	3	335	0.158*
	4	335	0.154*
	5	335	0.190*
Male	3	179	0.173*
	4	179	0.184*
	5	179	0.190*
Female	3	156	0.171*
	4	156	0.193*
	5	156	0.191*

Significance: * = $p < 0.001$

5.4 Descriptive statistics

Descriptive statistics including mean age, standard deviation, standard error, 95% confidence interval and age range per Phase by rib for male and female individuals are presented in Tables 5.2 and 5.3, and Figures 5.1 and 5.2 respectively. The descriptive statistics for the pooled sex data are listed in Appendix 2. A progressive increase in the mean age from Phases 0 to 8 is observed in male individuals, which differs according to rib number. For example, the fourth rib was found to exhibit morphological change (attain Phase 1) at an earlier mean age (18.16 ± 3.52 years) when compared to adjacent ribs (19.99 ± 3.95 years and 20.98 ± 5.12 years for ribs three and five respectively). Attainment of Phase 8 was variable, occurring earlier in rib five (60.17 ± 17.25 years) compared to ribs four (63.27 ± 11.85 years) and three (67.56 ± 8.84 years). The range of 95% confidence intervals increases by Phase 4 (and for all subsequent Phases) for all ribs examined. Female individuals exhibit Phase 1 characteristics at a later mean age when compared to males, with the attainment of Phase 1 occurring at the lowest mean age within the third rib (23.02 ± 7.26 years). In contrast to males, the fourth rib of female individuals was observed to display Phase 1 characteristics at the latest mean age (25.92 ± 9.97 years) of all ribs examined in this sample. Further, female individuals attained Phase 8 at a later mean age (between 68 to 71.97 years) when compared to males (60.17 to 67.56 years) across ribs three through five.

Table 5.2. Descriptive statistics according to Phase and rib for the male sample.

	Phase	N	Mean	SD	SE	95% CI	Age range
Rib 3	0	17	14.75	2.11	0.51	13.75-15.75	11-17
	1	44	19.99	3.95	0.59	18.82-21.16	12-28
	2	23	30.48	13.58	2.83	24.93-36.03	16-72
	3	34	33.58	9.43	1.62	30.41-36.75	22-56
	4	14	35.73	13.33	3.56	28.75-42.71	24-76
	5	13	52.77	18.75	5.20	42.58-62.96	27-80
	6	10	59.03	16.03	5.07	49.09-68.97	23-77
	7	14	57.51	14.69	3.93	49.81-65.21	31-79
	8	10	67.56	8.84	2.79	62.08-73.04	51-79
Rib 4	0	16	15.55	3.76	0.94	13.71-17.39	11-26
	1	35	18.16	3.52	0.60	16.99-19.33	12-28
	2	20	28.47	7.63	1.70	25.13-31.81	18-47
	3	38	28.67	6.98	1.13	26.45-30.89	19-53
	4	8	34.18	13.00	4.60	25.17-43.19	23-61
	5	17	46.73	17.04	4.13	38.63-54.83	22-80
	6	15	51.12	15.96	4.12	43.04-59.20	28-77
	7	15	60.29	16.91	4.37	51.73-68.85	23-79
	8	15	63.27	11.85	3.06	57.27-69.27	32-79
Rib 5	0	19	14.70	2.57	0.59	13.54-15.86	11-21
	1	48	20.98	5.12	0.74	19.53-22.43	14-39
	2	13	32.87	10.88	3.02	26.96-38.78	22-52
	3	29	30.65	9.14	1.70	27.32-33.98	19-61
	4	8	37.48	18.32	6.48	24.78-50.18	23-80
	5	14	43.51	15.56	4.16	35.36-51.66	27-77
	6	13	50.12	21.16	5.87	38.62-61.62	28-80
	7	21	58.38	12.19	2.66	53.17-63.59	33-77
	8	14	60.17	17.25	4.61	51.13-69.21	23-79
Total		179	34.81	19.12	1.43	32.01-37.61	11-80

Table 5.3. Descriptive statistics according to Phase and rib for the female sample.

	Phase	N	Mean	SD	SE	95% CI	Age range
Rib 3	0	16	14.04	3.23	0.81	12.46-15.62	10-21
	1	32	23.02	7.26	1.28	20.50-25.34	11-46
	2	23	31.28	12.01	2.50	26.37-36.19	19-66
	3	12	27.45	10.37	2.99	21.58-33.32	16-51
	4	14	38.31	11.13	2.97	32.48-44.14	23-61
	5	24	42.46	13.65	3.19	37.00-47.92	17-78
	6	18	49.63	16.21	3.82	42.14-57.12	18-78
	7	8	59.14	12.90	4.56	50.20-68.08	34-78
	8	9	71.67	7.15	2.38	67.00-76.34	56-78
Rib 4	0	17	14.88	4.57	1.11	12.71-17.05	10-23
	1	40	25.92	9.97	1.58	22.83-29.00	11-56
	2	20	29.03	9.37	2.10	24.92-33.14	15-51
	3	7	28.81	9.14	3.45	22.04-35.58	19-41
	4	11	30.95	7.30	2.20	26.64-35.26	16-41
	5	25	42.34	16.79	3.36	35.76-48.92	18-78
	6	13	48.78	17.79	4.93	39.11-58.45	21-78
	7	11	57.46	7.04	2.12	53.30-61.62	46-66
	8	12	70.03	8.34	2.41	65.31-74.75	56-78
Rib 5	0	14	13.14	2.76	0.74	11.69-14.59	10-18
	1	42	23.70	7.33	1.12	21.48-25.92	11-46
	2	14	26.96	7.84	2.09	22.85-31.07	16-41
	3	13	31.00	11.06	3.07	24.99-37.01	16-50
	4	12	33.92	9.40	2.71	28.60-39.24	21-51
	5	22	42.60	13.71	2.92	36.87-48.33	26-71
	6	19	50.40	16.16	3.71	42.13-57.67	20-78
	7	8	62.48	9.60	3.40	55.83-69.13	46-77
	8	12	68.00	10.71	3.09	61.94-74.06	27-51
Total		156	35.75	18.68	1.50	32.82 –38.68	10-78

Figure 5.1. Age distribution according to Phase for ribs three to five in the male sample. Boxes represent the median and interquartile range of estimated age per Phase, with whiskers indicating maximum and minimum estimated age values.

Figure 5.2. Age distribution according to Phase and ribs three to five in the female sample. Boxes represent the median and interquartile range of estimated age per Phase, with whiskers indicating maximum and minimum estimated age values.

5.5 Comparison of means

The following section comprises statistical tests to assess differences in estimated age across ribs three, four and five in the pooled and sex-specific samples. This is performed to evaluate the applicability of the Isçan et al Phase method to ribs other than (and inclusive of) the fourth in the Western Australian population.

5.5.1 One way repeated measures analysis of variance (ANOVA)

A one way repeated measures ANOVA was performed to assess whether estimated age (Phase score) varies between right ribs three through five. This test was performed in the pooled and individual sex samples. Mauchly's test of sphericity indicates that the data (pooled and sex specific) do not violate the assumption of normality (see Table 5.4). A significant difference between estimated age across ribs was found in the pooled-sex sample ($F(2,668) = 3.76$, $p < 0.05$, $\eta^2 = 0.011$) and in the male individuals ($F(2,356) = 9.519$, $p < 0.001$, $\eta^2 = 0.051$). The partial eta squared statistic for those results indicate a small and a moderate effect size for pooled sex and male differences respectively (Cohen 1988). There was no significant variation between ribs in the female sample ($F(2, 310) = 0.19$, $p = 0.827$, $\eta^2 = 0.001$).

Table 5.4. Mauchly's test of sphericity for pooled and sex specific Phase data across ribs three to five.

	Mauchly's W	Chi-square	df	Significance
Pooled	0.999	0.453	2	0.797
Male	0.988	2.055	2	0.358
Female	0.986	2.154	2	0.341

5.5.2 Paired samples t-test

Given the significant ANOVA result for the pooled-sex and male samples, a series of post hoc paired or dependent samples *t*-tests were performed to determine which ribs differed in mean estimated Phase. Estimated age (Phase) was found to significantly differ between ribs three (mean Phase 3.21, SD = 2.377) and four (mean Phase 3.41, SD = 2.514) ($t(334) = -2.488$, $p = 0.013$), and ribs three and five (mean Phase 3.38, SD =

2.567) ($t(334) = -2.185, p = 0.03$) within the pooled sex sample. The eta squared statistic for the difference between mean estimated age at ribs three and four (0.02) indicates a small to moderate effect, while the eta value for ribs three and five (0.014) indicates a small effect (Cohen 1988). A similar result occurs in the male sample, where mean estimated age significantly differs between ribs three (mean Phase 3.08, SD = 2.347) and four (mean Phase 3.47, SD = 2.475) ($t(178) = -4.5, p = <0.001$), and three and five (mean Phase 3.35, SD = 2.619) ($t(178) = -2.902, p = 0.004$). The eta squared result for difference in the mean estimated age between ribs three and four (0.102) and three and five (0.05) in the male sample suggest a large and moderate effect respectively (Cohen 1988). No other significant differences in mean age between rib pairs are evident.

5.5.3 Independent samples *t*-test

An independent samples *t*-test was performed to test for differences in the mean estimated Phase across ribs three, four and five between male and female individuals to establish any sex specific variation. Levene's test for equality of variance returned non-significant results for ribs three ($F = 2.432, p = 0.12$), four ($F = 2.037, p = 0.154$) and five ($F = 0.116, p = 0.733$), meaning that the assumptions of homogeneity of variance was not violated across the sample. No significant differences in mean Phase scores between males and females were found across ribs three ($t(333) = -1.128, p = 0.26$), four ($t(333) = 0.47, p = 0.639$) and five ($t(333) = -0.23, p = 0.818$) (see Table 5.5). The strength of the difference between means for ribs three, four and five was extremely small (eta square of 0.004, 0.007 and 0.002 for each respective rib) (Cohen 1988).

Table 5.5. Descriptive statistics for differences in the mean estimated Phase between ribs three, four and five in male and female individuals.

	Sex	N	Mean	SD
Rib 3	Male	179	3.08	2.347
	Female	156	3.37	2.408
Rib 4	Male	179	3.47	2.475
	Female	156	3.34	2.564
Rib 5	Male	179	3.35	2.619
	Female	156	3.42	2.514

5.6 Assessing relationships between variables

The following section presents the statistical tests used to assess the relationship between estimated age at different rib pairs, and the relationship between estimated and chronological age for each rib. This is followed by a series of linear regression analyses, from which age estimation standards are formulated.

5.6.1 Correlations

A bivariate Pearson's product moment correlation was performed to evaluate the association between estimated age across rib pairs (see Table 5.6), and estimated and actual age (see Table 5.7) in both the pooled and sex-specific samples. A strong, statistically significant positive correlation (Cohen 1988) occurred in all cases. In males, Phase scores from rib three correlate most strongly with those from rib four ($r = 0.885$, $p = < 0.001$), while in contrast, the opposite was found for female scores in this rib pair ($r = 0.780$, $p = < 0.001$). Phase scores in ribs four and five exhibited the strongest correlation within the female sample ($r = 0.825$, $p = < 0.001$). Male Phase scores were overall found to be more highly correlated with the actual age of individuals (mean coefficient of determination of 0.66, indicating that estimated age explained 66% of the variation in actual age), with rib four scores exhibiting the highest correlation to actual age ($r = 0.833$, $p = < 0.001$, 0.69 coefficient of determination). Female Phase scores accounted for approximately 63% of the variation in actual age, with rib five being most strongly correlated to chronological age ($r = 0.823$, $p = < 0.001$, 0.68 coefficient of determination). Of all the ribs examined, rib five scores were most strongly correlated with actual age in the pooled sex sample ($r = 0.809$, $p < 0.001$).

Table 5.6. Pearson correlation (*r*) between Phase scores (estimated age) across rib pairs.

Sex		Rib 3	Rib 4	Rib 5
Pooled (n = 335)	Rib 3	-	0.831*	0.842*
	Rib 4	0.831*	-	0.852*
	Rib 5	0.842*	0.852*	-
Male (n = 179)	Rib 3	-	0.885*	0.876*
	Rib 4	0.885*	-	0.878*
	Rib 5	0.876*	0.878*	-
Female (n = 156)	Rib 3	-	0.780*	0.806*
	Rib 4	0.780*	-	0.825*
	Rib 5	0.806*	0.825*	-

Significance (2 tailed): * = $p < 0.001$.

Table 5.7. Pearson correlation (*r*) between Phase scores (estimated age) and chronological age for each rib.

Sex	Rib no.	Chronological age	Coefficient of determination
Pooled (n = 335)	3	0.800*	0.640
	4	0.806*	0.650
	5	0.809*	0.655
Male (n = 179)	3	0.819*	0.67
	4	0.833*	0.693
	5	0.789*	0.623
Female (n = 156)	3	0.779*	0.607
	4	0.779*	0.607
	5	0.823*	0.677

Significance (2 tailed): * = $p < 0.001$.

5.6.2 Linear regression

Given the strong positive linear correlation between estimated and chronological age, a simple linear regression was performed to formulate a model to predict chronological age (outcome variable) using estimated Phase scores (predictor variable) from ribs three through five. The variables (raw Phase scores from ribs three, four and five) are submitted using the enter method, and results of the pooled and sex-specific regressions are presented in Table 5.8. Associated sex-specific scatterplots with regression lines for each rib are shown in Figures 5.3 to 5.5. It is evident that variation (spread of Phase scores) increases after Phase 0, particularly at Phases 5 and 6 for both sexes at ribs three through five. The age of male individuals was most accurately predicted based on rib four Phase scores ($SEE \pm 10.62$ years), followed by rib three ($SEE \pm 11.01$ years). Interestingly, the opposite was true for female individuals, where rib five Phase scores were most accurate in estimating chronological age ($SEE \pm 10.65$), followed by those derived from ribs three and four.

Table 5.8. Linear regression equations for the Isçan Phase estimation method for right ribs three through five in the Western Australian population.

Sex		N	R	R ²	F	Model equation	SEE (± years)
Pooled	Rib 3	335	0.800	0.64	591.66	ES _{CA} = 14.806 + 6.40 (R3)*	11.35
					7		
	Rib 4	335	0.806	0.65	617.14	ES _{CA} = 14.603 + 6.057 (R4)*	11.20
Male	Rib 5	335	0.809	0.655	632.38	ES _{CA} = 15.104 + 5.957 (R5)*	11.11
					0		
	Rib 3	179	0.819	0.67	359.94	ES _{CA} = 14.284 + 6.67 (R3)*	11.01
Female	Rib 4	179	0.833	0.693	399.90	ES _{CA} = 12.5 + 6.431 (R4)*	10.62
					4		
	Rib 5	179	0.798	0.637	311.18	ES _{CA} = 15.273 + 5.829 (R5)*	11.55
Pooled	Rib 3	156	0.779	0.607	238.23	ES _{CA} = 15.366 + 6.046 (R3)*	11.74
					4		
	Rib 4	156	0.779	0.607	237.53	ES _{CA} = 16.803 + 5.674 (R4)*	11.75
Male	Rib 5	156	0.823	0.677	322.89	ES _{CA} = 14.868 + 6.112 (R5)*	10.65
					8		
	Rib 3	156	0.779	0.607	238.23	ES _{CA} = 15.366 + 6.046 (R3)*	11.74
Female	Rib 4	156	0.779	0.607	237.53	ES _{CA} = 16.803 + 5.674 (R4)*	11.75
					8		
	Rib 5	156	0.823	0.677	322.89	ES _{CA} = 14.868 + 6.112 (R5)*	10.65
Pooled	Rib 3	156	0.779	0.607	238.23	ES _{CA} = 15.366 + 6.046 (R3)*	11.74
					4		
	Rib 4	156	0.779	0.607	237.53	ES _{CA} = 16.803 + 5.674 (R4)*	11.75
Male	Rib 5	156	0.823	0.677	322.89	ES _{CA} = 14.868 + 6.112 (R5)*	10.65
					8		
	Rib 3	156	0.779	0.607	238.23	ES _{CA} = 15.366 + 6.046 (R3)*	11.74
Female	Rib 4	156	0.779	0.607	237.53	ES _{CA} = 16.803 + 5.674 (R4)*	11.75
					8		
	Rib 5	156	0.823	0.677	322.89	ES _{CA} = 14.868 + 6.112 (R5)*	10.65

Significance = * p < 0.001.

Key: ES_{CA} = Estimated chronological age; R3 = Phase score for rib three; R4 = Phase score for rib four; R5 = Phase score for rib five.

Figure 5.3. Scatterplot with associated regression lines illustrating the association between Phase score and actual age (in years) for the third right rib. Iscan et al Phases correspond to the following mean age (in years): (1) 17.3;(2) 21.9;(3) 25.9;(4) 28.2;(5) 38.8;(6) 50;(7) 59.2 and (8) 71.5 in males: (1) 14;(2) 17.4;(3) 22.6;(4) 27.7;(5) 40;(6) 50.7;(7) 65.2 and (8) 76.4 in females.

Figure 5.4. Scatterplot with associated regression lines illustrating the association between Phase score and actual age (in years) for the third right rib. Isçan et al Phases correspond to the following mean age (in years): (1) 17.3;(2) 21.9;(3) 25.9;(4) 28.2;(5) 38.8;(6) 50; (7) 59.2 and (8) 71.5 in males; (1) 14;(2) 17.4; (3) 22.6;(4) 27.7; (5) 40; (6) 50.7;(7)65.2; (8) 76.4 in females.

Figure 5.5. Scatterplot with associated regression lines illustrating the association between Phase score and actual age (in years) for the third right rib. Iscan et al Phases correspond to the following mean age (in years); (1) 17.3;(2) 21.9;(3) 25.9;(4) 28.2;(5) 38.8;(6) 50;(7) 59.2 and (8) 71.5 in males;(1) 14;(2) 17.4;(3) 22.6;(4) 27.7;(5) 40;(6) 50.7;(7) 65.2 and (8) 76.4 in females.

5.6.3 Polynomial regression

Polynomial regression was performed to determine if the relationship between estimated and chronological age could be more accurately described using a curvilinear model, as growth and senescence is often not a linear phenomenon. The results of this analysis are shown in Table 5.9. A third order (cubic) polynomial was utilised for the pooled and individual sex samples.

The results of the polynomial models largely mirror those of the linear analysis; specifically the fourth rib produces the most accurate age estimate in males ($SEE \pm 10.59$ years), followed by scores obtained from the third ($SEE \pm 11.06$ years) and fifth ribs ($SEE \pm 11.61$). Phase scores from the fifth rib were most accurate for the estimation of age in females ($SEE \pm 10.54$ years), although unlike the results of the linear regression, Phase scores from the fourth rib ($SEE \pm 11.31$ years) were more accurate than those from rib three ($SEE \pm 11.47$ years). The use of polynomial regression to estimate age from rib Phase scores is associated with error rates between ± 11.07 to ± 11.32 years in this population, which is a small improvement over the use of linear regression equations (± 11.11 to ± 11.35 years). The greatest improvement in SEE scores between the results of the linear and polynomial regression occurred within the female sample where the mean SEE was reduced by 0.30 years

Table 5.9. Polynomial (cubic) regression equations for the estimation of chronological age (in years) from examined rib Phase scores for a Western Australian population.

Sex	Rib no.	N	R	R ²	F	p	Equation	SEE (±years)
Pooled	3	335	0.803	0.644	199.70	***	$ES_{CA} = 14.428 + 8.169(R3) - 0.832(R3)^2 + 0.084(R3)^3$	11.32
	4	335	0.812	0.660	213.85	***	$ES_{CA} = 16.68 + 4.94(R4) - 0.160(R4)^2 + 0.042(R4)^3$	11.07
	5	335	0.812	0.659	212.79	***	$ES_{CA} = 15.40 + 6.64(R5) - 0.48(R5)^2 + 0.05(R5)^3$	11.09
	3	179	0.819	0.671	118.99	***	$ES_{CA} = 14.64 + 5.64(R3) + 0.425(R3)^2 + -0.041(R3)^3$	11.06
	4	179	0.836	0.698	134.95	***	$ES_{CA} = 15.691 + 2.216(R4) + 1.13(R4)^2 + -0.081(R4)^3$	10.59
Male	5	179	0.798	0.637	102.56	***	$ES_{CA} = 15.321 + 5.811(R5) - 0.1(R5)^2 + 0.002(R5)^3$	11.61
	3	156	0.794	0.630	86.28	***	$ES_{CA} = 14.644 + 9.907(R3) - 1.764(R3)^2 + 0.178(R3)^3$	11.47
	4	156	0.800	0.640	90.20	***	$ES_{CA} = 17.029 + 8.593(R4) - 1.664(R4)^2 + 0.179(R4)^3$	11.31
	5	156	0.832	0.693	114.25	***	$ES_{CA} = 15.642 + 7.22(R5) - 0.883(R5)^2 + 0.104(R5)^3$	10.45
	3	156	0.794	0.630	86.28	***	$ES_{CA} = 14.644 + 9.907(R3) - 1.764(R3)^2 + 0.178(R3)^3$	11.47
Female	4	156	0.800	0.640	90.20	***	$ES_{CA} = 17.029 + 8.593(R4) - 1.664(R4)^2 + 0.179(R4)^3$	11.31
	5	156	0.832	0.693	114.25	***	$ES_{CA} = 15.642 + 7.22(R5) - 0.883(R5)^2 + 0.104(R5)^3$	10.45
	3	156	0.794	0.630	86.28	***	$ES_{CA} = 14.644 + 9.907(R3) - 1.764(R3)^2 + 0.178(R3)^3$	11.47
	4	156	0.800	0.640	90.20	***	$ES_{CA} = 17.029 + 8.593(R4) - 1.664(R4)^2 + 0.179(R4)^3$	11.31
	5	156	0.832	0.693	114.25	***	$ES_{CA} = 15.642 + 7.22(R5) - 0.883(R5)^2 + 0.104(R5)^3$	10.45

Significance = *** p < 0.001

Key: ES_{CA} = Estimated chronological age; R3 = Phase score for rib three; R4 = Phase score for rib four; R5 = Phase score for rib five.

5.6.4. Multiple linear regression

Multiple linear regression was performed to determine if age prediction accuracy could be increased by utilising multiple rib Phase scores. This was accomplished using both enter and stepwise approaches in the pooled and individual sex-samples. The results of the enter and stepwise linear regression models are presented in Tables 5.10 and 5.11 respectively

Female Phase scores produced the most accurate regression model for the estimation of chronological age using the enter method ($SEE \pm 9.81$ years, $F(3,152) = 136.557$), followed by pooled-sex ($SEE \pm 9.96$ years, $F(3,331) = 290.255$) and male scores ($SEE \pm 10.04$ years, $F(3,175) = 156.868$). Phase scores from all three ribs significantly contribute to the prediction of chronological age ($p < 0.001$). The use of a multiple regression equation reduced the SEE for all ribs and samples examined when compared to the results of linear and polynomial regression equations (see Tables 5.9 and 5.10). Approximately 72% of the variation in chronological age is accounted for by regression models using estimated Phase scores across all samples.

The results of the stepwise multiple regression (estimated Phase scores from ribs three through five are included or excluded based on the size of residuals) are largely the same as those achieved using the enter method; all three rib Phase scores produce the most accurate chronological age estimates ($SEE \pm 9.96$ years for the pooled sample and ± 9.81 years for the female sample). The use of Phase scores obtained exclusively from either the fourth or fifth ribs were associated with the highest error in the male ($SEE \pm 10.62$ years), pooled-sex ($SEE \pm 11.11$ years) and female ($SEE \pm 10.65$ years) samples respectively.

Table 5.10. Multiple linear regression equations (using the enter method or all three rib Phase scores) for the estimation of chronological age (in years) from examined rib Phase scores for a Western Australian population.

Sex	N	R	R ²	Adj. R ²	F	p	Equation	SEE (± years)
Pooled	335	0.851	0.725	0.722	290.255	***	ES _{CA} = 12.437 + (2.286 × R3) +	9.96
							(2.296 × R4) + (2.258 × R5)	
Male	179	0.854	0.729	0.724	156.868	***	ES _{CA} = 11.937 + (2.474 × R3) +	10.04
							(3.255 × R4) + (1.184 × R5)	
Female	156	0.854	0.729	0.724	136.557	***	ES _{CA} = 12.639 + (2.012 × R3) +	9.81
							(1.602 × R4) + (3.214 × R5)	

Significance = *** p < 0.001.

Table 5.11. Multiple linear regression equations (stepwise) for the estimation of chronological age (in years) for a Western Australian population.

Sex	Model	N	R	R ²	Adj. R ²	F	p	Equation	SEE (±years)
Pooled	1	335	0.809	0.655	0.654	632.380	***	$ES_{CA} = 15.104 + (5.957 \times R5)$	11.11
	2	335	0.839	0.704	0.703	395.636	***	$ES_{CA} = 13.232 + (3.295 \times R5) + (3.190 \times R4)$	10.30
	3	335	0.851	0.725	0.722	290.255	***	$ES_{CA} = 12.437 + (2.258 \times R5) + (2.296 \times R4) + (2.286 \times R3)$	9.96
Male	1	179	0.833	0.693	0.691	399.904	***	$ES_{CA} = 12.5 + (6.431 \times R4)$	10.62
	2	179	0.851	0.724	0.721	230.977	***	$ES_{CA} = 11.988 + (3.848 \times R4) + (3.078 \times R3)$	10.10
Female	1	156	0.823	0.677	0.675	322.898	***	$ES_{CA} = 14.868 + (6.112 \times R5)$	10.65
	2	156	0.846	0.716	0.712	192.619	***	$ES_{CA} = 12.972 + (2.574 \times R3) + (4.127 \times R5)$	10.02
	3	156	0.854	0.729	0.724	136.557	***	$ES_{CA} = 12.639 + (1.602 \times R4) + (2.012 \times R3) + (3.214 \times R5)$	9.81

Significance = *** p < 0.001.

Key: Pooled; Model 1 (Rib 5 scores), Model 2 (Rib 4 and 5 scores), Model 3 (Rib 3, 4 and 5 scores). Male; Model 1 (Rib 4 scores), Model 2 (Rib 3 and 4 scores). Female; Model 1 (Rib 5 scores), Model 2 (Rib 3 and 5 scores), Model 3 (Rib 3, 4 and 5 scores).

5.7 Transition analysis

Transition analysis was utilised to generate standards specific to a Western Australian population that describe the rate of morphological change that occurs within the sternal rib due to increasing age, from which age estimation standards can be derived. The results of this analysis for the pooled and individual sex-samples are shown in Table 5.12. Probability density plots that visually represent male and female data for the fourth rib are displayed in Figures 5.6 and 5.7 (pooled and sex-specific plots for the remaining ribs are presented in Appendix 3).

Initial age related metamorphosis of the fourth right rib was observed to occur at 12.73 years of age (± 6.36 years) within the pooled-sex sample. Male individuals were found to attain Phase 1 characteristics earlier than their female counterparts (12.99 years ± 5.61 years and 13.63 ± 6.3 years respectively) and continued to attain subsequent Phase characteristics prior to females until the transition between Phases 3 and 4, where this trend was largely reversed for remaining the Phases. The greatest difference between mean male and female age at transition occurs between Phases 7 and 8. Overall, the fourth rib was found to transition from Phase 0 to Phase 1 earlier than any other rib examined (14.11 ± 3.99 years of age at rib three and 14.59 ± 3.15 years of age at rib five).

In contrast to the latter findings in the fourth rib, female individuals transition from Phases 0 to 1 earlier when compared to male individuals for both the third and fifth ribs. Standard deviation scores across ribs and samples indicate widespread variability in the mean age at transition between Phases that begins at the transition between Phases 2 and 3. The probability density plots (Figures 5.6 and 5.7) demonstrate a progressive increase in mean age with increasing Phase. Lower standard deviations associated with the transition between Phases 0 and 1, and 1 and 2 in males, and Phases 0 to 1, and 7 to 8 in females, are represented by comparatively taller and narrower curves.

Table 5.12. Mean age at transition (in years) between Isçan et al Phases for ribs three, four and five in a Western Australian population.

Rib no.		Pooled sex		Male		Female	
	Phase	Mean	SD	Mean	SD	Mean	SD
Rib 3	0-1	14.11	3.99	14.53	3.07	13.90	4.93
	1-2	22.79	8.99	22.33	7.04	22.94	10.67
	2-3	30.65	15.10	30.30	13.40	30.94	15.99
	3-4	39.10	14.55	42.84	15.18	35.40	13.67
	4-5	45.38	15.25	48.99	14.26	41.87	16.69
	5-6	56.74	17.49	58.60	17.47	55.57	18.32
	6-7	67.47	17.84	67.39	19.90	68.21	15.35
	7-8	76.55	13.88	78.78	15.42	74.76	10.85
Rib 4	0-1	12.73	6.36	12.99	5.61	13.63	6.30
	1-2	23.03	11.63	20.75	4.37	25.51	16.71
	2-3	29.16	13.77	25.48	10.22	33.45	16.34
	3-4	36.27	12.22	36.82	11.08	35.90	15.00
	4-5	39.92	12.36	40.15	11.55	39.69	13.49
	5-6	52.55	16.31	51.29	16.72	54.46	16.75
	6-7	61.84	16.24	61.88	18.53	63.35	14.87
	7-8	74.25	17.24	78.52	22.30	68.64	7.89
Rib 5	0-1	14.59	3.15	15.30	2.61	14.02	3.37
	1-2	25.09	9.76	24.17	7.26	25.60	11.28
	2-3	29.29	11.87	28.17	13.72	30.12	11.00
	3-4	36.59	12.67	38.83	14.29	34.60	11.33
	4-5	40.72	13.20	42.55	14.93	39.15	11.55
	5-6	50.76	16.20	50.48	17.15	51.80	15.35
	6-7	61.45	17.00	58.71	17.93	64.90	12.72
	7-8	79.06	22.32	80.88	23.67	72.55	12.54

Figure 5.6. Probability density plot of male mean age at transition between Isçan age Phases 0 to 8 (fourth right rib).

Figure 5.7. Probability density plot of female mean age at transition between Isçan age Phases 0 to 8 (fourth right rib).

Chapter Six: Discussion and Conclusions

6.1 Introduction

The present study was primarily concerned with formulating age estimation standards specific to a Western Australian population based on the analysis of virtually reconstructed sternal ribs. The purpose of the final chapter of this thesis is to evaluate the significance of the results presented in relation to existing literature and the aims of the present project. A precision test to quantify the level of intra- and inter-observer concordance associated with Phase estimates was performed prior to primary data collection in order to determine if sternal ribs could be reliably assessed in clinical multi-slice tomography (MSCT) scans. Levels of concordance achieved in the present study are compared to those in the published literature for physical and virtual rib specimens, to assess the overall reliability of a MSCT based method. Inferential statistics used to examine the relationship between chronological and estimated age for the fourth right rib are discussed thereafter, including a critical comparison to standards presented by Isçan et al (1984b, 1985) and other global populations. Finally, the results of the regression and transition analyses performed for ribs three through five are considered in the context of the accuracy of existing adult age estimation methods. This is followed by a discussion of factors that may influence the timing, pattern and rate of morphological change due to senescence within thoracic structures. The chapter concludes with by considering of the limitations and forensic applications of the present study, and recommendations for future research.

6.2 Aim One: to determine whether sternal ribs can be reliably assessed in clinical multi-slice computed tomography (MSCT) scans

Given the relative novelty of the MSCT based method employed in the current study, and moderate to high levels of error known to be associated with the Isçan method (Hartnett 2010), the quantification of intra-observer error was considered to be particularly important for ensuring the accuracy and repeatability of results. The importance of error quantification is underscored by best practice principles recommended by the Scientific Working Group for Forensic Anthropology (SWGANTH) that require error appraisal and reporting to establish the legitimacy of a method.

Levels of intra- and inter-observer accordancy achieved in the current study are compared to those reported in the published literature for traditional and virtual methods alike, to determine the overall reliability of the Iscan method when applied to virtual rib reconstructions.

6.2.1 Intra- and inter-observer error

A precision test to quantify the level of intra- and inter-observer accordancy associated with Phase (age) estimates of the fourth right rib was performed prior to primary data analysis. Levels of intra- and inter-observer accordancy were found to be substantial ($K = 0.76$) and almost perfect ($K = 0.82$) respectively, according to recommendations published by Landis and Koch (1977). These levels of agreement suggest that sternal ribs can be reliably assessed when virtually reconstructed from MSCT scans, particularly when compared to error levels reported for studies that estimate age using physical bone specimens (see below). It is important to note that direct comparison between levels of error achieved in the current study with those reported in published literature is not always possible due to variations in what statistical test are used to quantify error, whether precision tests were performed prior to Phase analysis, and if physical or virtual rib specimens are utilised (if precision tests are even performed).

Levels of intra- and inter-observer accordancy achieved in the present study are comparable to those reported in the literature, where either physical or virtual rib specimens are assessed following the Iscan method. Salem et al (2014) reported substantial intra-observer agreement ($K = 0.73$ and $K = 0.71$) (Landis and Koch 1977) between Phase scores for 108 fourth right rib specimens originating from a Tunisian male population, evaluated over two occasions by two observers. Marginally higher (albeit still comparable) levels of intra-observer agreement have also been reported by Merritt (2014) ($K = 0.87$), who assessed a sample of 30 physical rib specimens derived from the William Bass Skeletal Collection. A comparable level of error between the virtual method used in the current study and traditional assessments based on the evaluation of physical rib specimens is particularly significant as it suggests that MSCT scans are a reliable alternate source of biological data that may be used in place of, or as an adjunct to, physical rib specimens.

Further, the results of the present study are also indirectly comparable to those achieved by Dedouit et al (2008), who assessed intra- and inter-observer concordance based on the evaluation of 39 MSCT reconstructions of the fourth right rib using Krippendorff's alpha reliability coefficient. Intra-observer error was 0.79 and inter-observer error ranged from between 0.78 to 0.86 (three observers). Krippendorff's alpha differs from Cohen's Kappa coefficient by measuring observed disagreement relative to disagreement that is expected by chance, and is applied to two (or more) observations by two (or more) operators (Zapf et al 2016). Calculation of Krippendorff's alpha produces values between -1 and 1, where -1 indicates inverse, and 1 perfect agreement (Zapf et al 2016). While Kappa and Krippendorff coefficient values cannot be directly compared due to differences in their underlying statistical assumptions and applications, it is worthwhile to note that levels of both intra- and inter-observer concordance achieved in the present study are within the range reported by Dedouit et al (2008). This is significant as the Dedouit et al study represents one of the only known published attempts to estimate age from the fourth rib using MSCT reconstructions.

Variation in the levels of intra- and inter-observer error achieved between different studies can be (in part) attributed to the morphoscopic nature of adult age estimation methods, and the qualitative data these produce. Commonly employed adult age estimation techniques rely on the subjective visual analysis of skeletal traits and the degeneration of skeletal morphology as a function of age, meaning that estimates are affected by the experience, training and familiarity of the observer with the method, and are thus inherently associated with variations in intra- and inter-observer error (Hefner 2002; Hefner & Stephen 2012). For example, Isçan et al (1986a) found individuals without Doctoral degrees to be less accurate (mean deviation of 1.06 Phases from the ideal) in estimating the age of male fourth right rib specimens, as compared to those with Doctoral degrees (mean deviation of 0.92 Phases). The opposite was found for female rib specimens (mean deviation of 0.72 Phases for judges without degrees and 0.85 for those with degrees) (Isçan et al 1986b), meaning that personal differences in the perception of morphological features and interpretation of Phase descriptions may also be a source of potential variation. Phase descriptions have been criticized for requiring significant interpretation with regard to the quality and weight

of bone, and the inclusion of descriptive states such as 'becoming' and 'lighter than,' especially because observers are unlikely to have access to physical bones representative of each Phase to compare with a specimen of interest (Hartnett 2010). While evaluation of bone density and quality may not be directly applicable to virtually reconstructed specimens, revisions to the Phase descriptions that describe the texture and physical appearance of ribs have been shown to improve the accuracy of age estimates in older individuals (Merritt 2014). Finally, variation in levels of intra- and inter-observer error can be attributed to differences in MSCT instrumentation, reconstruction processes and the medium used to assess rib specimens (virtual versus physical) between studies, although further research is required to quantify these differences given the relative paucity of MSCT derived rib studies.

6.3 Aim Two: to determine if age related metamorphosis of the fourth right sternal rib follows the same pattern of progression in a Western Australian population relative to the original method

The importance of utilising population specific standards for the compilation of the biological profile has been established by previous research, which demonstrates that the use of geographically or temporally disparate standards decreases the level of accuracy associated with estimates of age, sex, stature and ancestry for an unknown individual (Kimmerle et al 2008; Franklin et al 2013a; Lottering 2013; Ubelaker & DeGaglia 2017). The use of contemporary population specific standards is advised by the SWGANTh, and has achieved widespread acceptance among the community of forensic anthropologists as best practice to ensure the accuracy of a particular analysis (Franklin et al 2013a; Lottering 2013). Differences in the overall rate of senescence and appearance of morphological characteristics at different age Phases between the Caucasian American population studied by Isçan et al (1984b, 1985) and other global populations have been noted by several subsequent studies (e.g. Oettle & Steyn 2004; Salem et al 2014; Yavuz et al 1998). The results of fourth rib Phase analysis achieved in the present study are compared to those presented by Isçan et al (1984b, 1985) to assess the overall accuracy and applicability of the method relative to the Western Australian population. Mean ages at each Phase are compared to those for other global populations to assess geographical variation in the timing of rib senescence.

6.3.1 Comparison of the results of the present study to Isçan et al. (1984b, 1985)

The results of the present study (see Table 6.1) are largely comparable to those reported by Isçan et al (1984b) for the male sample. Initial morphological change (amorphous pit indentation and increase in rim irregularity signifying Phase 1) is demonstrated to occur at approximately the same mean age in both populations (between 17 and 18 years). Given that Isçan reported that the mean age for Phase 1 is based on the analysis of only four individuals, no definitive conclusions concerning the age at which rib degeneration occurs in males can be drawn, as a sample this small is unlikely to be representative of the scope of biological variation in the Isçan population, and lacks statistical power (Button et al 2013). Statistically underpowered studies are associated with falsely inflated measures of effect size and low rates of reproducibility, making it difficult to generalise results to a wider population (Button et al 2013; Suresh & Chandrashekhara 2012). Males within the Western Australian population demonstrate delayed degeneration in fourth rib morphology across Phases with the exception of Phase 8, where characteristics associated with this Phase were observed approximately eight years earlier than that reported by Isçan et al (1984b). This difference may be due to the inclusion of a 32-year-old individual in Phase 8 (lowest age reported by Isçan for Phase 8 is 44 years). The mean age for Phase 8 is 65.52 years when this individual was removed, meaning that the application of the Isçan et al standards would result in the overestimation of age, as Western Australian individuals exhibit Phase 8 characteristics approximately six years earlier than their American counterparts.

Increased variation in the Western Australian sample (wider 95% confidence intervals and age ranges per Phase) can be attributed to the inclusion of older individuals in earlier Phases and *vice versa*, particularly for Phases 2 through 5 and Phase 7. CT scans representing these individuals could not be excluded on the basis of poor resolution, and were included in the final statistical analysis, as the observed rib morphology was considered to potentially reflect normal biological variation. The reported mean ages for Phases 2 through 5 and Phase 7 were recalculated excluding individuals at either the maximum (Phases 2 to 5; 47, 53, 61 and 80 years of age, respectively) or minimum (Phase 7; 23 years of age) end of the age range presented for each Phase; this was performed to determine if these values affected the mean age values presented in Table 6.1. The revised mean ages are presented in Table 6.2, which show that the removal of individuals considered to be outliers did not alter the overall trend of rib degeneration observed in the Western Australian population, as evidenced by the revised mean ages being greater at each Phase (except for Phase 8) compared to those reported by Isçan.

The results obtained for female individuals in the present study are comparable to those reported by Isçan et al (1985) from Phase 4 onwards. Initial degeneration in rib morphology (Phase 1) was observed to occur approximately 11 years after that reported by Isçan in the Western Australian population. It is unlikely that this result represents a meaningful difference, as the mean age provided by Isçan is based on the evaluation of a single individual. Comparison between mean ages at Phase 1 is thus inconsequential, as the Isçan reported age is not statistically valid (see above). The female mean age at Phase 1 in the present study (25.92 years) is significantly higher than that reported for males (18.16 years), possibly as the result of a 56-year-old individual being assessed into Phase 1 (see below).

Table 6.1. Sex specific mean age and deviation of stage attainment between the present study and Işcan et al (1984b, 1985).

Phase	Males					Females				
	N	Mean age	SD	95% CI	Range	N	Mean age	SD	95% CI	Range
Işcan et al.										
1	4	17.3	0.50	16.5 – 18.0	17 - 18	1	14.0	N/A	N/A	N/A
2	15	21.9	2.13	20.8 – 23.1	18 - 25	5	17.4	0.68	15.5 – 19.3	16 – 20
3	17	25.9	3.50	24.1 – 27.7	19 - 33	5	22.6	0.74	20.5 – 24.7	20 – 24
4	12	28.2	3.83	25.7 – 30.6	22 - 35	10	27.7	1.46	24.4 – 31.0	24 – 40
5	14	38.8	7.00	34.4 – 42.3	28 - 52	17	40.0	2.96	33.7 – 46.3	29 – 77
6	17	50.0	11.17	44.3 – 55.7	32 - 71	18	50.7	3.52	43.3 – 58.1	32 – 79
7	17	59.2	9.52	54.3 – 64.1	44 - 85	16	65.2	2.81	59.2 – 71.2	48 – 83
8	12	71.5	10.27	65.0 – 78.0	44 - 85	11	76.4	2.66	70.4 – 82.3	62 - 90
Present study										
1	35	18.16	3.52	16.99 – 19.33	12 – 28	40	25.92	9.97	22.83 – 29.00	11 – 56
2	20	28.47	7.63	25.13 – 31.81	18 – 47	20	29.03	9.37	24.92 – 33.14	15 – 51
3	38	28.67	6.98	26.45 – 30.89	19 – 53	7	28.81	9.14	22.04 – 35.58	19 – 41
4	8	34.18	13.00	25.17 – 43.19	23 – 61	11	30.95	7.30	26.64 – 35.26	16 – 41
5	17	46.73	17.04	38.63 – 54.83	22 – 80	25	42.34	16.79	35.76 – 48.92	18 – 78
6	15	51.12	15.96	43.04 – 59.20	28 – 77	13	48.78	17.79	39.11 – 58.45	21 – 78
7	15	60.29	16.91	51.73 – 68.85	23 – 79	11	57.46	7.04	53.30 – 61.62	46 – 66
8	15	63.27	11.85	57.27 – 69.27	32 – 79	12	70.03	8.34	65.31 – 74.75	56 – 78

Females within the Western Australian sample exhibit delayed degeneration in fourth rib morphology until Phase 7, where degeneration occurred approximately eight and six years earlier in Phases 7 and 8 respectively when compared to mean ages reported by Isçan. This may be (in part) due to differences in sampling and access to rib specimens obtained from older individuals, as the oldest female individual included in the present study is 12 years younger than that reported by Isçan. As with the male sample, increased levels of variation in the female sample can potentially be attributed to the inclusion of older individuals in earlier Phases and *vice versa*. Individuals at the maximum end of the age range reported for Phases 1 to 3 (56, 51 and 41 years of age respectively), and the minimum end of the range reported for Phases 4 to 6 (16, 18 and 21 years of age respectively), were excluded to determine if these values affected the mean age reported for these Phases in Table 6.1. Revised mean ages for Phases 1 to 6 are displayed in Table 6.3. The mean age for Phases 1 and 2 reported in the present study was decreased when older individuals were removed, although are still higher than those reported by Isçan and for other global populations (see section 6.3.3), potentially as a result of individuals in their 30s and 40s estimated to Phase 1 or 2. Removal of individuals considered to be outliers did not alter the trend observed in mean ages presented in Table 6.1, as female individuals displayed delayed rib degeneration from Phases 1 to 6, and accelerated degeneration for Phases 7 and 8.

6.3.2 Progression of age related sternal rib metamorphosis

Age related change in the morphology of sternal ribs in both male and female samples followed the rate and pattern of degeneration described by Isçan et al (1984b, 1985). Scalloping of the rim and development of the pit from an amorphous indentation to a V then U shaped cavity was observed in all specimens, although scalloping was not as regular or obviously pronounced in some cases, possibly due to the tendency of the three dimensional reconstructive processes to smooth surface irregularities (see Section 6.7). No differences in the depth and shape of the pit between sexes was observed. Morphological change in the male sample was characterised by the development of bony projections at the superior and inferior end of the rim at Phase 4, which became increasingly prominent with advancing age. Female rib specimens were observed to develop a central arc within anterior and/or posterior rim walls, although the plaque like deposits that line the pit floor in Phase 5 and beyond were

not observed in the present study. These deposits either do not develop in Western Australian individuals, or were unable to be visualised using the imaging protocol. Limitations associated with scan resolution and CT slice thickness are unavoidable as clinical CT scans currently represent the only source of population specific biological data available for a Western Australian population (see Section 6.5.2). Window formations within rib walls were observed in both male and female specimens assigned to Phase 8, although the latter was not frequently observed.

Table 6.2. Revised mean age and range for Phases 2 through 5 and Phase 7 following removal of outliers for the male sample.

Phase	Revised mean age	Revised age range
1	18.16	12-28
2	27.50	18-43
3	28.01	19-47
4	30.35	23-47
5	41.55	22-61
6	51.12	28-77
7	62.95	39-79
8	65.52	51-79

Table 6.3. Revised mean age and range for Phases 1 through 6 following removal of outliers for the female sample.

Phase	Revised mean age	Revised age range
1	24.46	11-46
2	28.10	15-46
3	26.72	19-37
4	32.44	24-41
5	43.35	22-78
6	53.83	28-78
7	57.46	39-79
8	70.03	56-78

6.3.3 Population variation in sternal rib morphology

The results of the present study are compared to those reported for Turkish (Yavuz et al 1998), Indian (Meena et al 2012, 2013) and Hungarian populations (Wolff et al 2012) (see Tables 6.4 and 6.5) to identify variations in the timing of sternal rib end metamorphosis. Revised mean ages and age ranges for male and female individuals (see Tables 6.2 and 6.3) have been used for comparative purposes, as they are more representative of the overall mean per Phase, and do not alter the general trend of delayed rib degeneration observed in original data. It is important to note that the mean ages presented for the above studies discussed in this section are based on the physical analysis of the fourth rib.

The mean age per Phase for male and female individuals in the present study is overall very similar to those of other global populations, particularly Turkish (Yavuz et al 1998) and Indian (Meena et al 2012). It is difficult to determine how comparable Western Australian mean ages are to those presented for a Hungarian population for Phases 1 to 3, as the latter results are based on extremely small samples. Similar Phase mean ages across Western Australian, Indian and Turkish populations (specifically for Phases 1 to 7 in males and 4 to 8 in females) suggests that degeneration in rib morphology follows the same pattern and rate of progression in all three populations. This result is somewhat surprising, as Turkish and Indian populations rank lower than Australian populations on measures such as health status, income, wealth and general subjective well-being, which are associated with lower socioeconomic status (OECD 2017). Individuals of low socioeconomic status are known to experience delayed skeletal growth during puberty, which results in diminished skeletal maturity at adulthood (see Section 6.5.1). Definitive statements concerning the effect that socioeconomic status has on skeletal degeneration in the aforementioned studies cannot be made, as this has not been previously explored, and data pertaining to health status and general well-being is not recorded as it is not considered to be medically relevant within Western Australia (see Section 6.5.1).

The greatest variation in reported mean age between populations occurs at Phases 2 and 8 for males and Phases 1 to 3 in females. The mean age obtained for Phase 2 for Western Australian males (27.5 years) is more comparable to that reported for

Hungarian males (23 years of age), although this is likely to be the result of individuals in their 30s and 40s estimated as Phase 2 in the present study. The underestimation of individuals in their 30s and 40s in the female Western Australian sample is also likely to account for the variation in the mean age observed in Phases 1 to 3. Western Australian individuals exhibit Phase 8 morphology at least five years earlier compared to their Turkish and Indian counterparts, which may be due to population specific genetic factors that influence the degeneration of skeletal morphology, and the prevalence of degenerative disorders associated with old age that influence bone remodelling (Manolagas & Parfitt 2010). The effect of these factors (among others) on rib aging is yet to be examined. While the standards reported by Yavuz et al (1998) are comparable to those presented by Meena (2012, 2013) and the present study, it is important to note that the Yavuz et al data are relatively outdated and may not be representative of rib ageing in contemporary Turkish populations. Comparable mean ages per phase to other global populations for both male and female samples used in the present study is significant as it reinforces the utility of virtual rib specimens in lieu of physical bones.

Table 6.4. Mean age per Phase for the fourth right rib following Isçan et al standards for male individuals in different global populations.

Phase	Present study			Turkish			Indian			Hungarian	
	N	Mean age	Range	N	Mean age	Range	N	Mean age	Range	N	Mean age
1	35	18.16	12-28	13	17.9	17-19	5	17.4	17-18	1	22.0
2	19	27.50	18-43	18	21.1	20-23	10	21.6	19-28	3	23.0
3	37	28.01	19-47	13	27.2	24-29	11	26.1	20-30	2	28.5
4	7	30.35	23-47	19	30.2	28-33	11	28.5	26-35	11	38.6
5	15	41.55	22-61	38	39.9	34-44	9	39.1	30-55	27	50.2
6	15	51.12	28-77	23	49.3	45-53	20	49.7	42-73	20	67.5
7	14	62.95	46-79	11	57.5	53-65	19	59.2	48-75	18	71.3
8	14	65.52	51-79	7	75.3	66+	14	70.2	52-85	N/A	N/A

Table 6.5. Mean age per Phase for the fourth right rib following Iscan et al standards for female individuals in different global populations.

	Present study			Turkish			Indian			Hungarian	
Phase	N	Mean age	Range	N	Mean age	Range	N	Mean age	Range	N	Mean age
1	38	24.46	11-46	7	15.4	13-18	3	14.33	14-15	2	25.0
2	19	28.10	15-46	34	18.4	17-19	4	17.50	17-19	1	24.0
3	6	26.72	19-37	15	21.7	20-24	12	22.90	20-30	2	26.5
4	10	32.44	24-41	35	28.3	25-32	12	28.40	25-40	3	55.3
5	24	43.35	22-78	29	40.3	33-48	9	38.70	33-50	12	53.8
6	11	53.83	28-78	14	54.4	49-57	7	50.20	36-68	2	69.0
7	11	62.95	39-79	8	63.8	57-71	7	63.20	59-72	8	75.9
8	12	70.03	56-78	1	76.0	72+	8	74.87	70-80	N/A	N/A

6.4 Aim Three: to develop Western Australian forensic standards for the estimation of age from the sternal rib

Age estimation standards based on the analysis of the sternal rib specific to a contemporary Western Australian population do not currently exist, as the data required to compile such standards has not been available. This is problematic for forensic practice as the SWGANTH recommends the use of population-specific standards to estimate age, and a paucity of available data seriously restricts conclusions that can be made about the biological profile of an unidentified adult or elderly individual. Further, deficiencies in the availability of predictive models to estimate chronological age from Phase scores relative to the fourth right rib are present in the existing literature. The final aim of the present study thus sought to address the aforementioned gaps in the literature by utilising regression and transition analyses to formulate age estimation models based on ribs three through five. Further, morphological variations between right ribs three, four and five are discussed with respect to variations documented in the literature to assess the practicality of applying fourth rib standards to adjacent ribs. The predictive accuracy of the results of the present study are then compared to those associated with other adult age estimation methods that utilise similar statistical approaches.

6.4.1 Regression analysis

Adult age estimation methods are based on the analysis of the progressive degeneration of skeletal and dental morphology with age, and are inherently associated with lower accuracy rates in comparison to methods for the juvenile skeleton due to the variable effect of individualistic health and lifestyle factors that influence skeletal degeneration within and between individuals (Franklin 2010, Karkhanis et al 2014). In a forensic context, the accepted error threshold for adult age estimation methods is ± 10 years (Willems et al 2002). Regression analysis was used in the present study to formulate predictive age models associated with statistically quantifiable measures of accuracy, as measured by the standard error of the estimate (SEE).

The SEE statistic is not reported for all commonly employed morphoscopic adult estimation techniques that perform regression analysis (accuracy is instead reported in terms of inaccuracy and bias or not at all) possibly due to a lack of standardisation in statistical reporting among practicing forensic anthropologists (Garvin and Passalacqua 2012). The results obtained for the various regression analyses performed in the present study are compared to those for the dentition and specialised skeletal and dental age estimation techniques from Australia and other contemporary global populations. These studies were chosen for comparison purposes as they include individuals of similar ages to the present study, and report the accuracy of predictive regression models using the SEE.

A small improvement in the SEE between the linear and polynomial regression models was observed, with the latter being more accurate overall. Relatively large SEE values across both regression methods (between ± 10.62 and ± 11.55 years for males and ± 10.65 and ± 11.75 years for females using linear regression; and between ± 10.59 and ± 11.61 years for males and ± 10.45 and ± 11.47 years using polynomial regression) suggest that the relationship between estimated and chronological age is not well represented by either a linear or cubic model. However, it stands to reason that older individuals would most likely be placed in higher Phases, which is suggestive of a positive linear relationship between estimated and chronological age. Thus, the linear regression would at face value be expected to produce more accurate results. This discrepancy may be due to the presence of 'outliers' or individuals whose age was significantly over- or under-estimated relative to their chronological age, which diminish the overall accuracy of the prediction model due to the statistical sensitivity of regression analysis (Pallant 2011). Individuals that were identified as outliers were not removed as those scans did not meet exclusion criteria, and are potentially indicative of meaningful biological variations within the sample population. More extensive research is required to determine the accuracy of linear predictive models and quantify whether these variations represent meaningful biological differences in the sample population.

Considered collectively the results of the transition analysis demonstrate that greater accuracy (lower SEE values) in the prediction of chronological age is achieved when Phase scores from multiple ribs (preferably all three in the current study) are used (± 10.04 years for male and ± 9.81 years for female individuals). The age prediction accuracy of stepwise and enter multiple regression methods was the same (± 9.96 years for the pooled sample, and ± 9.81 years for the females) when Phase scores from all three ribs were analysed collectively. The stepwise regression based on the male sample failed to generate a predictive model utilising Phase scores from all of the ribs examined (rib five scores excluded), possibly on the basis of greater residual values associated with the fifth rib. This is supported by the results of the linear and polynomial regressions, where scores obtained from the fifth rib alone produced higher SEE values (± 11.55 and ± 11.61 years respectively) compared to ribs three and four in males. The multiple regression equation produced using the enter method could be used in place of a stepwise regression in this case as it is associated with a lower level of error (± 10.04 years) compared to the combined use of Phase scores from ribs three and four (± 10.10 years). As the accuracy of the multiple regression equations are comparable, either stepwise or enter methods could realistically be used for age estimation in a forensic context, albeit depending on the availability and quality of the ribs.

The SEE of the linear and multiple regression analyses performed in the present study are compared to those for a Western Australian and other global populations based on specialised skeletal and dental age estimation techniques including pulp chamber dimensions and pulp tooth ratios (e.g. Bosmans et al 2005; Zaher et al 2011; Kanchan-Talreja et al 2012; Karkhanis et al 2013, 2014; Misirlioglu et al 2014), aspartic acid racemization of dentin (e.g. Elfawal et al 2015; Fu et al 1995; Griffin et al 2008), and bone histology (Table 6.6) (e.g. Kim et al 2007; Han et al 2009; Stout et al 1994; Watanbe et al 1998b). The results of the present study are largely comparable to those reported for other adult age estimation techniques, particularly for dental estimation methods in an Indian and Western Australian population. Karkhanis et al (2013) (not shown in Table 6.6) reported accuracy rates between ± 8.062 and ± 10.507 years for females and ± 8.212 and ± 10.074 years for males using a linear regression model to estimate age, which was reduced to ± 6.827 and ± 7.893 years for females and ± 6.306

and ± 8.683 years in males when multiple scores from the mandibular molars were used. This result reflects the trend observed in the present study where multiple regression models produced more accurate (lower SEE) age estimates. Given that chronological age can be estimated from the ribs with a comparable level of accuracy to methods that already exist for a Western Australian population, the results of the current study provide a valuable source of data from an alternative skeletal element that can be used to estimate the age of living individuals, in lieu of, or in addition to, the dentition.

The reported SEE values for the present study are significantly higher when compared to specialised age estimation techniques such as aspartic acid racemization and bone histology, possibly due to the quantitative rather than qualitative nature of those methods. While amino acid racemization is an accurate and well established forensic age estimation technique, racemization studies are based on relatively small samples with a limited age range (e.g. Elfawal et al 2015); this clearly limits the generalizability of results to other populations due to low statistical power. The invasive nature of this technique also precludes it from being readily used to estimate the age of living individuals, unless the analysis can be performed following necessary dental treatment (extraction, albeit this is unlikely). Further, the rate of amino acid racemization is known to be influenced by temperature, pH level, post-mortem taphonomy, the region and type of tooth that is chosen for analysis, and whether dentin or enamel is used (Masters 1986; Ohtani 1995; Waite et al 1999). Contemporary, population specific racemization data is lacking, probably due to the level of expertise and the cost of specialised equipment required to carry out these analyses, as well as the availability of tooth samples (Franklin 2010). Similarly, histological methods cannot be used to estimate the age of living individuals, and while these techniques produce age estimates within the threshold required for forensic purposes, reported accuracy is known to be influenced by the sex and age distribution of cadaver samples used to produce the predictive models (Kim et al 2007). Other acknowledged sources of variation that may account for differences in reported SEE values include choice of bone (relating to the biological function and hence rate of remodelling), staining medium and the use of single versus multifactorial methods (Han et al 2009; Watanabe et al 1998b).

Table 6.6. Accuracy (SEE) of various dental and histological adult age estimation techniques using regression analysis (multiple regression unless stated otherwise).

Method		N	Age range (years)	Population	SEE (years)
Dental (Kvaal method)	Bosmans et al 2005	197	19-75	Belgium	± 9.5 for all 6 teeth in pooled sex sample
	Kanchan-Talreja et al 2012	100	25-77	Indian	± 11.90 to ± 12.08 (depending on viewing technique)
	Misirlioglu et al 2014	114	17-72	Turkish	± 5.74 for females and ± 6.60 for males (3 teeth)
	Karkhanis et al 2014	279	20-73	Western Australian	± 7.963 (pooled sex sample)
Dental (Pulp cavity index)	Zaher et al 2011 (linear regression)	144	12-60	Egyptian	± 1.36 to ± 5.08 for central and ± 1.2 to ± 2.70 for lateral incisors
	Karkhanis et al 2013	450	9-60	Western Australian	± 6.827 to ± 7.893 (females); ± 6.306 to ± 8.683 (males)

Method		N	Age range (years)	Population	SEE (years)
Aspartic acid racemization	Fu et al 1995	28	14-69	Chinese	± 1 to ± 4
	Griffin et al 2008 (linear regression)	31	3-60	English	± 8.7 reduced to ± 6.2 when <35 years old
	Elfawal et al 2015	89	10-31	Kuwaiti	± 1.26 reduced to ± 1.12 using validation sample
Bone histology	Stout et al 1994 (rib)	60	11-88	Caucasian	± 10.43 using osteon population density (OPD); ± 7.182 using OPD and rib Phase score
	Watanbe et al 1998b (femur)	98	<1-92	Japanese (cadaver)	± 6.393 (stepwise) and ± 4.884 (enter) multiple regression
	Kim et al 2007 (rib)	64	20-67	Korean	± 4.821 (known sex) and ± 4.971 (unknown sex)
	Han et al 2009 (femur)	72	35-94	Korean	± 6.650

6.4.2 Transition analysis

Transition analysis was used in the present study to augment the results of multiple regression analysis by calculating the mean age at which right ribs three to five transition between Phases. The use of this method in contemporary reference standards is increasing, as it is known to overcome limitations, such as age mimicry and excessively large age ranges associated with an estimate of age (Boldsen et al 2002). Transition analysis has been used to model age related morphological change in the medial clavicle, spheno-occipital synchondrosis and pubic symphysis in Australian populations (Bassed et al 2010, 2011; Franklin & Flavel 2014, 2015; Lottering et al 2013).

The results of the transition analysis demonstrate comparable mean ages at transition between Phases across ribs and male and female samples. The fourth rib was found to transition between Phases 0 and 1 earlier than any other rib in both the male and female sample, with males undergoing transition at an earlier age compared to females (12.99 ± 5.61 years compared to 13.63 ± 6.30 years). The opposite was found for the higher Phases, with male individuals transitioning between Phases 7 and 8 at a later mean age (78.52 ± 22.30 years) when compared to females (68.64 ± 7.89 years). Earlier female transition between Phases 7 and 8 also found to occurred for ribs three and five. In general, the sternal rib of female individuals was found to undergo morphological change earlier when compared to males, particularly at Phases 0 to 1 and 7 to 8 (see Table 5.12).

The standard deviation values across Phases and samples demonstrate a high level of variability in the timing of morphological change. A significant increase in standard deviation values occurs at the transition between Phases 2 and 3 in males, and Phases 1 and 2 in females. Although the results of the current transition analysis cannot be directly compared to the mean age reported for each Phase by Isçan (1984b, 1985), it is interesting to note that the mean age of transition from Phases 0 to 1 for the fourth rib in males and females was lower than the mean age reported by Isçan for both samples at Phase 1 (17.3 and 14 years of age for males and females respectively), meaning that the use of the Isçan standard in a Western Australian population would result in the overestimation of age for individuals within this Phase. The opposite is

true of older Phases for all ribs across the male and female sample where the mean age at transition in the Western Australian population is higher than mean ages reported by Isçan. The results of the transition analysis suggest that standards developed for the fourth right rib can be used to estimate age from adjacent ribs. Further research on the age of transition between age Phases using larger samples and geographically disparate populations is required to compare and quantify the results of the present study.

6.4.3 Analysis of ribs three and five

Isçan et al (1984b) concluded their seminal work by hypothesising that minimal variation in the morphology of ribs three to five is likely to exist, meaning that standards developed for the fourth right rib could be used to estimate the age of adjacent ribs. This hypothesis has been supported by Loth et al (1994) (see below), leading to the use of fourth ribs standards for ribs three and five in validation studies of the Isçan method (e.g. Matrilie et al 2007; Merritt 2014). However, existing literature that evaluates intercostal variation between right ribs three to five is scarce, and does not comprehensively examine differences according to ancestry. Yoder et al (2001) found Phase scores between right ribs two and four to differ significantly in a sample of bone specimens derived from Caucasian American individuals, while only Phase scores at the fourth right rib and the third left rib differed significantly in an African American sample. The results of that study demonstrate the importance of using population-specific data to determine whether standards from the fourth right rib can be applied to other rib specimens. In the present study, fourth rib Phase ageing standards were applied to virtual reconstructions of right ribs three and five, to determine if chronological age could be reliably estimated by evaluating the strength of the relationship between Phase estimates at different rib pairs.

A significant difference in estimated Phase scores was observed for ribs three and four, and three and five in the male sample. These differences were found to be strongly correlated across rib pairs ($r = 0.885$ between ribs three and four, and $r = 0.876$ between ribs three and five). This result suggests that significant differences in the morphology of ribs three to five exist in male individuals, meaning that due caution should be applied when using male fourth rib standards to estimate the age of

adjacent ribs, particularly the third right rib. The statistically significant difference reported in the current study represents the first instance of observed Phase discrepancies between right ribs three through five in the current literature. Loth et al (1994) estimated the age of ribs three and five to the same Phase as the fourth right rib in 79 per cent of cases, and to within one Phase of the fourth rib in all remaining cases. Similarly, Yoder et al (2001) reported no significant differences in the estimated age of ribs two to eight in the right series for a Caucasian male sample. Differences in estimated Phase scores between the present study and those reported in the existing literature could potentially be explained by differences in the analysis of ribs (traditional versus virtual methods; see below), although more research is required to corroborate the results of the present study.

No significant difference in estimated Phase scores across ribs was observed for the female sample, meaning that the Isçan et al (1985) Phase aging standard can be used to estimate the age of right ribs three and five in a Western Australian female population. This is consistent with Loth et al (1994) who noted minimal intercostal variation between ribs three, four and five for female individuals (rib morphology across different specimens was judged as belonging to the same Phase in 76% of cases, and the age of ribs three and five was estimated to a single Phase of rib four in 91% of cases). However, the results of the Loth et al study (1994) are based on the analysis of a very small sample (33 individuals), which limits the ability to generalise results to the broader population. In contrast, significant differences ($p = 0.027$) in Phase scores for right ribs four and five in Caucasian females have been reported by Yoder et al (2001). It is important to note that these studies are based on the analysis of physical rib specimens, and comparative data concerning the quantification of intercostal differences in the right rib series using virtual techniques does not currently exist. Further research is thus needed to determine if comparable differences or similarities between estimated Phase scores across ribs can be achieved between traditional and virtual methods.

Variation in the morphology of adjacent ribs is likely to occur as the result of differences in the rate of rib growth and remodelling within and between individuals, and the amount of mechanical loading that different ribs are subjected to as the result

of respiration and movement of the upper body (Loth et al 1994; Yoder et al). Differences in the morphology of ribs between males and females (as observed in the present study) have been attributed to sex-specific (sexually dimorphic) adaptations of the thorax that facilitate respiration due to differential energy requirements, and the female need to house a foetus during pregnancy (Garcia-Martinez et al 2016). These differences could also be explained by individual variation, or the effect of individualistic lifestyle and general health factors such as occupation, diet, physical activity and chronic disease (Isçan et al 1984b, 1985). Differences in endocrine function are also suggested as a source of variation between the sexes (Isçan et al 1984b), although its specific effect on rib morphology due to senescence has not been comprehensively investigated. Further research is again required to determine the impact that the aforementioned factors have on rib morphology, and what effect this may have (if any) on the accuracy of the Phase ageing method.

6.5 Potential sources of variation in the present data

The following section addresses the main sources of potential variation in the data, specifically relating to the effect of socioeconomic status (SES) of individuals included in the sample, and the resolution of the CT scans on the accuracy of skeletal reference standards produced in the present thesis.

6.5.1. Socioeconomic status

The genetically pre-determined potential for skeletal growth and attainment of skeletal maturity is known to be mediated by a suite of environmental factors, including the socioeconomic status of a given individual (Schmeling et al 2000). SES is measured relative to access to a variety of resources (e.g. education, adequate nutrition and medical care), in addition to the overall quality of their living environment (e.g. levels of psychological stress, chronic disease, overcrowding as a result of living in large family groups) (Bradley & Crowyn 2002; Delemarre van de Waal 1993; Franklin & Flavel 2015). Low SES has been demonstrated to slow skeletal growth during puberty, resulting in diminished skeletal maturity in adulthood (Delemarre-van de Waal 1993). The application of skeletal reference standards from a population of higher SES to that of a lower status thus results in the underestimation of age. The SES of individuals included in the present study is not known; such information is not

considered to be medically relevant and legislative guidelines for Ethical Conduct in Human Research (NHMRC) restrict the collection and distribution of patient information. It can be assumed that all individuals of the sample population had reasonable access to medical care as they presented at public hospitals within Western Australia, although it is not possible to make definitive statements as to the effect that other factors such as nutrition or quality of living conditions have on variation in sternal rib morphology. Given that the SES of individuals included in studies performed by Isçan were similarly not recorded, it is not possible to determine whether the age of individuals in the current study was under- or over-estimated as a result of SES.

6.5.2 The partial volume effect

The resolution of the scans evaluated in the present study constitutes another source of variation in the data. Slices were obtained at a thickness of 0.8 mm at an increment of 0.4 mm, and ranged between 0.625 mm and 1.7 mm. The slice thickness of CT scans varies according to the composition of the anatomical structure of interest; the level of detail that can be obtained and reconstructed to form a virtual three-dimensional model decreases with increasing slice thickness (Ford & Decker 2016). This phenomenon is referred to as the partial volume effect and it has been identified in the literature as a being a potential source of misclassification between successive stages of skeletal development (Franklin & Flavel 2015). Muhler et al (2006) evaluated CT scans of the medial clavicular epiphysis of 40 different individuals at various slice thickness intervals (1, 3, 5 and 7 mm) to estimate age according to the degree of epiphyseal ossification. Clavicular ossification was determined in all scans obtained, although stage classification was found to be dependent on slice thickness (higher ossification stages were assigned to scans with greater slice thickness). In a forensic context, misclassification to advanced stages of skeletal development results in the over-estimation of age, and potentially exposes the individual concerned to unnecessary legal action in cases requiring the age of a living individual to be estimated. As such, the authors recommend a maximum slice thickness of 1 mm be used for the purpose of forensic age estimation from the clavicle (Muhler et al 2006). Further, a maximum slice thickness of 1.25 mm has been recommended by Ford & Decker (2016) as a means of preserving fine anatomical details, such as cranial sutures and dentition.

The tendency towards obtaining thick-slice CT scans in clinical practice is due to the need to prevent exposing living individuals to excessive levels of radiation according to the principle of ALARA (As Low as is Reasonably Achievable), and requirements imposed by medical ethics (Milenkovic et al 2014). It is not currently possible to overcome the challenges that thicker slices and subpar CT resolution pose to the accuracy of age estimation standards in Western Australia, as post-mortem scanning of human remains is not performed, and clinical CT scans represent the only way to access population specific data. Despite the trade-off between slice thickness and resolution, clinical CT scans remain a valuable source of biological data that can be utilised in lieu of skeletal collections and foreign reference standards to ensure the accuracy of age estimates by providing access to contemporary, population specific data.

6.6 Forensic applications

Skeletal reference standards for biological profiling of unknown individuals are sparse in Australia in general, owing to a relatively small population, low homicide rates, and a lack of skeletal reference collections (Donlon 2016), meaning that practitioners must rely on data formulated from American or European populations. As previously discussed, the use of foreign standards decreases the accuracy with which aspects of the biological profile can be estimated, and potentially exposes living individuals to unnecessary legal ramifications in cases where chronological age is contested (Franklin et al, 2015b). The use of a virtual analysis protocol based on the evaluation of clinical MSCT scans eliminates the need to rely on skeletal collections as a source of data, allowing contemporary, population specific standards free of the influence of age mimicry (tendency of standards to mimic composition of skeletal reference collections) to be generated, thereby increasing the amount and accuracy of data available for analysis in a forensic context.

The age estimation models produced in the present thesis are directly applicable to a Western Australian population specifically, and potentially the broader Australian population generally. Predictive models using multiple rib Phase scores were accurate to within ± 10.04 years for males and ± 9.81 years for females, which is comparable to existing standards for Western Australia and other global populations, and within the

accepted accuracy for adult estimation methods generally (Willems et al 2002). The current project further validates the utility of MSCT as a credible modality for age estimation from the sternal ribs and adds to the existing body of literature that establishes the applicability of virtual methods in forensic practice. Virtual sources of biological data have been utilised in mass disaster scenarios in Australia for the purposes of individual identification and resolution of commingled remains (O'Donnell et al 2011), in addition to being used as an adjunct to traditional autopsy procedures (Blau et al 2008). The provision of predictive age models based on multiple ribs by the current study is potentially advantageous for mass disaster scenarios, or the analysis of remains exposed to taphonomic agents where damage to ribs may have occurred.

6.7 Potential limitations

Time and resource constraints limited the final sample to 335 individuals (179 male and 156 female). This sample may not necessarily be representative of the Western Australian population as a whole, as the individuals examined represent only a small portion of the general population presenting at hospitals during the time of CT scan acquisition. Another potential limitation associated with the sample population is the lack of documentation of ancestry related information and the general health and disease profile of individuals. Age related morphological change and the rate and pattern of overall aging of the fourth right rib is known to vary according to ancestry (Isçan et al 1987; Oettle & Steyn 2000; Salem et al 2014), or lifestyle, health and disease factors (e.g. occupation, diet, amount of physical activity and chronic disease); these are suggested to be potential contributors to the aging process (Isçan et al 1984b; Isçan et al 1986a, 1986b). Further, bilateral variation in rib morphology was not assessed in the present study due to time constraints. Previous research, however, suggests that bilateral differences in the sternal ribs are minimal (Yoder et al 2001), although this could not be confirmed in the present study.

The use of a virtually reconstructed thorax precludes age estimation by means of tactile examination of rib specimens. The Isçan et al method emphasises evaluating the general condition of rib specimens in relation to their weight, density, porosity and texture at each Phase. While these characteristics are important to the overall process of age estimation using physical rib specimens, they cannot be evaluated using clinical

CT reconstructions. This potentially places the accuracy of CT reconstructions at a disadvantage to the more traditional method of analysis. Loth (1995) stated that the 'feel' (density and solidity of bone) is an important discriminatory factor for the estimation of age, as ribs that exhibited greater firmness than that expected based on their appearance were assigned to a younger Phase. The inability to physically palpate specimens to determine other important features (such as the sharpness of the rib rim or edge) that might be difficult to discern (see below) is another disadvantage of using three-dimensional reconstructions. While these limitations are unavoidable given the virtual basis of the method, the results of the multiple linear regression (see above) demonstrate that age can be accurately and reliably estimated from virtual rib specimens.

Further, certain rib features listed by Işcan et al are more difficult to assess compared to others due to MSCT post-imaging reconstruction processes. For example, the thickness and degree of sharpening of the rib wall that is used to differentiate Phases 4 and above for males and females is particularly difficult to discern, as the 3D reconstruction smooths the appearance of any sharp or irregular rib projections, potentially resulting in the underestimation of age of individuals in Phase 4 and over (Dedouit et al 2008). As per the recommendations of Dedouit et al (2008), wall thickness was further examined using the 2D orthogonal view available in *OsiriX* in cases where the thickness could not be established in the 3D view alone. A similar limitation applies to the determination of the porosity of bone in relation to the set contrast of the scan. The appearance of the costochondral junction is obscured when the contrast is too high (unable to sufficiently visualise the rib edge and rim wall) and conversely, portions of the wall and pit floor disappear when the contrast is too low. A certain amount of observer discretion is therefore required to ensure that features of the rib are visualised in a manner that is as representative of the physical rib as possible. The plaque like deposit that lines the pit during Phase 5 for females was likewise difficult to distinguish given the limited ability to visualise porosity in CT reconstructions.

6.8 Future directions

Given the relative novelty of the transposition of traditional methods of skeletal analysis to virtual reconstructions, future research would benefit from the replication of the method using a larger, more ethnically diverse sample that is more evenly distributed, particularly within older age groups. Future studies utilising a larger sample drawn from all States and Territories of Australia are required to establish whether aging of the sternal rib varies based on geographic location, or if the standards developed in the present thesis are sufficient to estimate the chronological age of individuals nationwide. It would also be beneficial to explore the existence of any bilateral variation that occurs as part of the aging process within a Western Australian population, in order to increase the amount of morphological data available for the modelling of senescence within thoracic structures. Several studies have documented bilateral dissimilarities in the process of sternal rib aging (e.g. Loth et al 1994; Yoder et al 2001), although research in this area is scarce and has not specifically been performed for an Australian population. Additional data collected from different global populations using virtual techniques may also aid in validating the method and increasing the amount of data available for the construction of population specific standards.

The clinical nature of the present study and the use of virtual rib specimens does not allow inter-method error to be evaluated (age estimation of the same rib specimens using both physical and virtual examination techniques). The quantification of inter-method error permits comparison between levels of inter- and intra-observer error, the overall accuracy of the Isçan method, and its efficacy when evaluated using different analytical platforms (Dedouit et al 2008). This limitation is difficult to overcome given the use of living individuals, although it could potentially be resolved using 3D printing. These techniques utilise medical imaging modalities such as MSCT scans to produce 3D anatomical prototypes based on triangular mesh data that are generated from an area of interest during the post- processing of DICOM images (Rengier et al 2010). These 3D prototypes are primarily used in medicine for the purposes of education, surgical planning and construction of patient specific medical implants or tissue scaffolds (Chia & Hu 2015; Rengier et al 2010), although such models

have also been used to reconstruct and analyse skeletal trauma in archaeological and forensic contexts alike (Fantini et al 2008; Kettner et al 2011).

Finally, the overall accuracy and reliability of the Iscan method would benefit from future studies that attempt to quantify age related changes in rib morphology in relation to shape variants, with (for example) geometric morphometric techniques. Such techniques facilitate shape changes to be quantified numerically with rigorous statistical methods (Mitteroecker & Gunz 2009). Nikita (2013) attempted to quantify shape differences in the morphology of the sternal ribs throughout life using geometric morphometric techniques. That study found no differences in rib shape and morphology between fourth right rib specimens under 35 years of age when compared to specimens over the age of 60 (see Chapter Three for a detailed review). This result is counter intuitive, as the rib is documented to undergo obvious changes in its overall appearance across populations, particularly over the age of 60 (Iscan et al 1984b, 1985, Oettle & Steyn 2000). The unexpected result obtained by Nikita (2013) may be due to errors in sampling, the use of discrete over semi-landmarks, and an ineffective digitising protocol. When used in combination with MSCT skeletal reconstructions, virtual geometric morphometric techniques eliminate the need to use a physical digitiser that may damage fragile diagnostic features (such as bony projections or rim irregularities) and could potentially overcome some of the limitations associated with that study. In addition, the use of semi-landmarks over discrete landmarks may also limit problems associated with intra-observer error and increase the accuracy with which age estimates are made. Further research in this area is required to determine if changes in rib morphology can be numerically quantified and analysed to produce more accurate age estimates using rib specimens.

6.9 Conclusions

The present thesis presents a novel set of skeletal reference standards for the estimation of age from the fourth right rib, with demonstrated comparable levels of intra- and inter-observer accordance to that published in the existing literature (e.g. Dedouit et al 2008; Merritt 2014; Salem 2014) and a similar level of predictive accuracy to other Western Australian standards (Karkhanis et al 2013, 2014) and other global populations (Bosmans et al 2005; Griffin et al 2008; Kanchan-Talreja et al 2012).

The application of the Phase ageing method in a Western Australian population yielded comparable results to that originally reported by Iscan et al (1984b, 1985) for Phases 4 and beyond, and were found to be applicable for estimating age in right ribs three and five. The use of multiple regression equations utilising Phase scores from right ribs three, four and five produced the most accurate estimates of chronological age (within ± 10.04 years for males and ± 9.81 years for females). The results of the present study further validate the utility of MSCT scans as an analogue for skeletal reference collections or physical dry bone specimens and demonstrate that techniques based in virtual analyses can be used to reliably estimate age in a Western Australian population.

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Appendices

Appendix 1

THE UNIVERSITY OF
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Human Ethics

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Our Ref: RA/4/1/4362

02 May 2016

Associate Professor Daniel Franklin
Centre for Forensic Science
MBDP: M311

Dear Professor Franklin

HUMAN RESEARCH ETHICS OFFICE – AMENDMENT REQUEST APPROVED

Novel approaches to the forensic identification of human remains: bone morphometrics

Student(s): Siobhan Lee Sullivan, Jacqueline Mary Elizabeth Noble, Eden Centaine Johnstone-Belford, Amanda Jane Barville, Janae Marie Barnes, Jessica Laura-Anne Laurier, Nina Joan Throsby, Charise Nanielle Baker, Magdalena Klaudia Blaszkowska

I confirm receipt of your correspondence requesting an amendment to the protocol for the above project.

Approval has been granted for the amendment as outlined in your correspondence and attachments (if any) subject to any conditions listed below.

The following is a brief description of the amendment and any conditions that apply:

1. Addition of following students to the project: Amanda Barville, Janae Barnes, Jessica Laurier, Nina Throsby, Charise Baker and Magdalena Blaszkowska.

If you have any queries, please contact the HEO at humanethics@uwa.edu.au.

Please ensure that you quote the file reference RA/4/1/4362 and the associated project title in all future correspondence.

Yours sincerely

Dr Caixia Li
Manager, Human Ethics

Appendix 2

	Phase	N	Mean	SD	SE	95% CI	Age range
Rib 3	0	33	14.40	2.69	0.47	13.48-15.32	10-21
	1	76	21.27	5.74	0.66	19.98-22.56	11-46
	2	46	30.88	12.69	1.87	27.21-34.55	16-73
	3	46	31.98	9.94	1.47	29.11-34.85	16-56
	4	28	37.02	12.12	2.29	32.53-41.51	23-76
	5	37	46.09	17.29	2.84	40.52-51.66	17-80
	6	28	52.99	16.50	3.12	46.88-59.10	18-78
	7	22	58.11	13.77	2.94	52.36-63.86	32-79
	8	19	69.51	8.14	1.87	65.85-73.17	51-79
Rib 4	0	33	15.21	4.14	0.72	13.80-16.62	10-26
	1	75	22.30	8.56	0.99	20.36-24.24	11-56
	2	40	28.75	8.44	1.33	26.13-31.37	15-51
	3	45	28.69	7.24	1.08	26.57-30.81	19-53
	4	19	32.31	9.09	2.27	28.22-36.40	16-61
	5	42	44.11	16.82	2.60	39.02-49.20	18-80
	6	28	50.03	16.55	3.13	43.90-56.16	21-78
	7	26	59.09	13.49	2.65	53.90-64.28	23-79
	8	27	66.28	10.81	2.08	62.20-70.36	32-79
Rib 5	0	33	14.04	2.72	0.47	13.11-14.97	10-21
	1	90	22.25	6.36	0.67	20.94-23.56	11-46
	2	27	29.80	9.72	1.87	26.13-33.47	16-53
	3	42	30.75	9.64	1.49	27.83-33.67	16-61
	4	20	35.34	13.34	2.98	29.49-41.20	21-80
	5	36	42.95	14.24	2.37	38.30-47.60	26-77
	6	32	50.28	18.03	3.19	44.03-56.53	20-80
	7	29	59.51	11.52	2.14	55.32-63.70	33-77
	8	26	63.79	14.87	2.92	58.07-69.51	23-79
Total		335	35.25	18.89	1.03	33.23-37.27	10-80

Appendix 3

Pooled probability density plots

Rib 3

Rib 5

Sex specific probability density plots
Males

Rib 3

Rib 5

Females
Rib 3

Rib 5