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Skeletal age estimation and the epiphyseal scar challenging the status quo

Davies, Catriona Mairead

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Skeletal Age Estimation and the Epiphyseal Scar: Challenging the s*tatus quo*

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Abbreviations and Definitions

AGFAD – Arbeitsgemeinschaft für Forensische Altersdiagnostik (Working group for forensic age diagnostics)

ANOVA – Analysis of Variance

A-P – Anterior – Posterior Plane

BMD – Bone Mineral Density

BMI – Body Mass Index

CSF – Colony Stimulating Factor

CT – Computed Tomography

DMPA – Depot Medroxyprogesterone Acetate

DOB- Date of Birth

DOI – Date of Image Acquisition

DRUJ – Distal Radioulnar Joint

DXA – Dual X-Ray Absorptiometry

FDA – Federal Drug Administration (of the United States of America)

GH – Growth Hormone

GLM – General Linear Model

HRT – Hormone Replacement Therapy

IGF – Insulin-like Growth Factor

Ihh – Indian Hedgehog

IL – Interleukin

JPEG – Joint Photographic Experts Group

M-L – Medial – Lateral Plane

MMPs - Metalloproteinases

MRI – Magnetic Resonance Imaging

OPG – Osteoprotegerin

PACS – Picture Archiving and Communication System

PBM – Peak Bone Mass

PHV – Peak Height Velocity

PTH – Parathyroid Hormone

PTHrP – Parathyroid Hormone related Protein

RC – Rotator Cuff

RGD – Arginine-glycine-aspartic acid tri-peptide

UK – United Kingdom

URN – Unique Reference Number

TGF – Transforming Growth Factor

TNF – Tumour Necrosis Factor

TPR – Total Persistence Rate (The percentage of a given population in whom a Persistence Score of >0 was recorded.)

TPS – Total Persistence Score (The sum of the individual scores assigned to each of the six tracks according to the criteria outlined in the method)

VEGF – Vascular Endothelial Growth Factor

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Firstly, I would like to thank my supervisors Professor Sue Black and Dr Lucina Hackman for their input throughout this process and to whom I owe a great debt of gratitude. Without their advice and support, this project would not have been possible. It is thanks to the belief that they have shown in me throughout the past three years that I am able to submit this work. I can only hope that I have, at least in part, fulfilled their expectations.

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Finally, I would like to thank my family, in particular my parents, for their support, reassurance and understanding throughout not only this project, but throughout my time at the University of Dundee. I truly could not have done it without them and it is to them that I dedicate this work.

Declaration

I, Catriona Mairead Davies, declare that I am the sole author of this thesis and that I have undertaken the work, of which this thesis is a record. Unless otherwise stated, all references cited in this work have been consulted by me. No portion of the work on which this thesis is based has been previously submitted for a higher degree at this, or any other, institution.

Signed ..... Date .....

Catriona M Davies BSc (Hons)

Summary

In recent years, the discovery of fragmented human remains has garnered significant attention from the national and international media, particularly the recovery of multiple lower limbs and feet from coastlines in North America. While cases such as these stimulate public curiosity, they present unique challenges to forensic practitioners in relation to the identification of the individual from whom the body part originated.

Many researchers have attempted to apply characteristics or morphologies of the podiatric skeleton to the assessment of living stature or biological sex; however relatively few studies relating the development and morphology of the foot to skeletal age estimation have been undertaken. Of these studies, only one has been tested on a population other than that on which it was based. In addition to the absence of validation studies, some maturity criteria against which skeletal development may be gauged, such as the persistence or obliteration of the epiphyseal scar, have yet to be supported by empirical data.

In response to the deficiencies in the literature relating to skeletal age estimation from the foot and ankle and the possible persistence of the epiphyseal scar in adult individuals, a two phase study was devised. The initial phase consisted of a test of two radiographic approaches to skeletal age estimation from the juvenile foot and ankle. Utilising a collection of radiographs of the juvenile foot and ankle obtained from female and male individuals between birth and 18 years of age, the accuracy of the two approaches were tested. This study showed that while a good correlation was observed between the chronological age of the individuals and the estimated age according to the radiographic atlas; the alternative scoring system approach was deemed not appropriate for use in skeletal age estimation.

The second phase of this study consisted of an analysis of the persistence of epiphyseal scars in five anatomical locations in adult females and males between 20 and 50 years of age. Through statistical analysis, the relationships between the level of persistence of the epiphyseal scar and chronological age, biological sex and side of the body were assessed. Analyses showed that the level of persistence or obliteration of epiphyseal scars varies throughout the skeleton and within

individual skeletal areas. Although some of this variation may be attributable to the biological sex of the individual, the overall relationship between chronological age and the level of persistence or obliteration of the epiphyseal scar was not found to be of sufficient strength to support a causative link. It was found that the complex interactions of multiple factors including those localised to specific skeletal areas explains a larger proportion of the variation in the persistence of the epiphyseal scar than biological sex alone. Based on these findings, it is proposed that the level of persistence or obliteration of the epiphyseal scar encountered may be influenced by the degree of mechanical loading to which the area is subjected.

This study marks the introduction of a new paradigm in relation to the persistence of the epiphyseal scar in adult individuals and presents a significant argument against the application of the persistence or obliteration of the epiphyseal scar as a maturity criterion in skeletal age estimation.

Hypotheses

Hypothesis 1: The standards of skeletal age estimation applicable to the region of the foot and ankle in juvenile individuals are appropriate, accurate and of sufficient reliability to be utilised, in the forensic context, for the estimation of age of an individual from a modern Scottish population.

Hypothesis 2: The epiphyseal scars of the proximal humerus, distal radius, distal femur, proximal tibia and distal tibia will become obliterated soon after the completion of epiphyseal fusion, resulting in radiographically unremarkable bone.

Aims and Objectives

Prior to the commencement of this study, two research aims were identified. These were:

1. To test two radiographic standards applicable to age estimation from the juvenile foot and ankle on a sample of radiographs from a modern population from North-East Scotland.

2. To examine the validity of the application of the epiphyseal scar as a radiographic maturity criterion in skeletal age estimation through the assessment of the persistence or obliteration of this feature in five anatomical regions in a sample of radiographs from a modern population from North-East Scotland.

A number of objectives were set to facilitate the attainment of the stated research aims:

1. To obtain access to, and collect, a sample of radiographic images of the foot and ankle from female and male children aged between birth and 20 years of age.

2. To undertake assessments of age on a sample of radiographs of the foot and ankle using two radiographic approaches to age estimation

3. To perform a statistical analysis of the age estimations undertaken using two methods of assessment and assess their reliability and accuracy in the context of the estimation of chronological age from radiographic images.

4. To assess the repeatability of the methods of skeletal age assessment through intra-observer and inter-observer testing.

5. To collect radiographs (both anterior-posterior and medial-lateral views) from five anatomical areas (both left and right sides): the proximal humerus, the distal radius, the distal femur, the proximal tibia and the distal tibia from female and male individuals aged between 20 and 50 years of age.

6. To devise a scoring system to assess the level of persistence of the epiphyseal scar in each of five anatomical regions.

7. To perform statistical analyses of the relationships between chronological age, biological sex and side of the body and the observed persistence of the epiphyseal scar in each of five anatomical regions.

8. To assess the repeatability of the scoring system in each anatomical area through devising an intra-observer and inter-observer test.

9. To compare the persistence of epiphyseal scars between anatomical areas.

10. To compare the persistence of the epiphyseal scar in three discrete regions of each bone in each anatomical area.

1 Literature Review

1.1 Estimation of skeletal age

The earliest formation of the osseous skeleton in humans begins in the clavicle at approximately 6 weeks of intrauterine development (Ogata and Uhthoff, 1990). The process of ossification continues into the third decade when, for example, the fusion of the medial clavicular epiphysis is completed (Scheuer and Black, 2000). The majority of the skeleton however will have attained adult morphology some years previously. The pattern and timing of the maturation of some areas of the skeleton is closely correlated with chronological age and a result, the term "skeletal age" has been adopted to relate the maturational progress of the skeleton to the passage of time (Ritz-Timme *et al.*, 2000).

Although, in a developmentally normal individual, the parameters of skeletal age and chronological age are closely related, they are not synonymous. The concept of charting the relationship between skeletal maturation and chronological age has been widely applied in the clinical monitoring of normal paediatric growth, most notably by Tanner and Whitehouse (1976) in the production of paediatric growth charts. In the context of human identification, it is the strength of the relationship between skeletal and chronological age which enables the practitioner to assign an estimate of age at death to human remains; however the strength of the relationship between skeletal maturation and chronological age is not constant throughout life or between skeletal regions. The reliability of the estimation of skeletal age is therefore dependent on whether the remains are skeletally mature or immature and to which region of the skeleton they belong (Ritz-Timme *et al.*, 2000; Scheuer, 2002).

Through monitoring the progressive appearance, growth and maturation of various skeletal regions in individuals of known chronological age, a series of standards were published relating to the development of the juvenile skeleton (Greulich and Pyle, 1959; Hoerr *et al.*, 1962; Tanner *et al.*, 1962; Pyle and Hoerr, 1969; Tanner *et al.*, 1975; Tanner *et al.*, 2001). These texts have since been used to estimate the chronological age of individuals based on their stage of skeletal development as devised from radiographic images.

Since the publication of these original studies, the literature relating to juvenile age estimation has been enhanced through the testing of existing methods and the development of additional approaches to skeletal age estimation , including the application of alternative medical imaging modalities including computed tomography (CT) (Schulz *et al.*, 2005; Kellinghaus *et al.*, 2010); Magnetic Resonance Imaging (MRI) (Dvorak *et al.*, 2007a; 2007b; Dedouit *et al.*, 2012) and ultrasound (Castriota-Scanderbeg *et al.*, 1998; Bilgili *et al.*, 2003; Mentzel *et al.*, 2005; Khan *et al.*, 2009; Schmidt *et al.*, 2013).

Although the chronological age of an individual is absolute, estimated skeletal age is dependent on a number of factors, not least the method through which the assessment of age is conducted. There is evidence within the literature that when conducted using medical imaging techniques, the estimated age may vary from that assigned through the gross inspection of dry bone (Cardoso, 2008a; 2008b). This is due to the continued alteration to trabecular morphology following the completion of external fusion which, although visible using medical imaging, is obscured in a dry bone specimen. It should also be noted however that estimations of chronological age may vary between methods of medical imaging. As a result, in the final stages of skeletal maturation, estimations of chronological age may differ depending on the modality used (Castriota-Scanderbeg *et al.*, 1998). It is therefore imperative that the approach taken in the estimation of age is compatible with that in which the skeleton is examined.

As this study was conducted solely using x-ray images, only radiographic methods of age estimation will be considered in this section.

1.1.1 Estimation of age from skeletal morphology

Characteristics considered in the assessment of skeletal development in relation to chronological age include the appearance; overall size and morphology of individual bones; the proximity between centres of ossification including epiphyses and their respective diaphyses; and the stage of epiphyseal fusion observed (Workshop of European Anthropologists, 1980; Scheuer, 2002). As an individual ages and skeletal elements attain their adult morphology, the number of potential sources of information on which an estimation of age may be based

decreases and the importance of the remaining sites of maturation to skeletal age estimation increases (Ritz-Timme *et al.*, 2000; Rösing *et al.*, 2007).

Within the skeletal regions commonly used in age estimation, the final demarcation of adulthood is often classified as the fusion of the epiphyses to their diaphyses, the completion of which indicates attainment of adult morphology and size for each bone (O'Connor *et al.*, 2008). Although a bone may appear skeletally mature on gross examination, it has been suggested that the presence of a radio-opaque line in the location of the former growth plate is an indication that epiphyseal fusion has recently occurred (Todd, 1930; 1937). The obliteration of this feature, termed the "*epiphyseal scar*", is presumed to occur as a result of bone remodelling which progressively alters the underlying trabecular structure (Garden, 1961; O'Connor *et al.*, 2008). As bone remodelling is a process which continues throughout the life of the individual, the obliteration of the epiphyseal scar was presumed to be a time-linked process. This has led to the obliteration of the epiphyseal scar being employed as a maturity criterion in a number of methods of skeletal age estimation (Schmeling *et al.*, 2004; Schulz *et al.*, 2005; Schmidt *et al.*, 2008; Baumann *et al.*, 2009; Kellinghaus *et al.*, 2010; Bassed *et al.*, 2011; Garamendi *et al.*, 2011).

Examination of the relevant literature has failed to locate any research which explicitly supported the use of the persistence or obliteration of the epiphyseal scar as a criterion in skeletal age estimation. To the contrary, a study by Baumann *et al*. (2009) found that although a minimum age could be assigned to the obliteration of the epiphyseal scar in the distal radius, no conclusions could be drawn regarding the relationship between the presence or obliteration of the feature and increasing chronological age. This highlights the need for the validity of methods of skeletal age assessment and the criteria on which they are based, to be tested.

The requirement for testing this assumption has been reinforced by observations made through forensic casework, where interpretation of the presence of epiphyseal scars in the proximal and distal tibia and distal femur as indicators of

recent epiphyseal fusion in a male individual, led to an underestimation of age by approximately 10 years (see Appendix A).

1.1.2 Validity of methods of skeletal age estimation

The requirement for scientific validity is of particular importance if, through the application of methods of age assessment, an estimation of age is to be provided in a forensic context. According to the recommendations made by The Law Commission of England and Wales (2011), it is incumbent upon researchers and practitioners to ensure that the methods used in their assessments meet the criteria for judicial admissibility. This includes the requirement that the methods are reliable, a criterion that can only be satisfied through repeated testing. Although methods of age estimation from several skeletal regions including the knee (Hackman and Black, 2013a); hand and wrist (Andersen, 1971; Vignolo *et al.*, 1992; Bull *et al.*, 1999; Groell *et al.*, 1999; Haiter-Neto *et al.*, 2006; Schmidt *et al.*, 2007a; Lynnerup *et al.*, 2008; Büken *et al.*, 2009; Hackman and Black, 2013b); and elbow (Sauvegrain *et al.*, 1962; Brodeur *et al.*, 1981; Canavese *et al.*, 2008) have been subjected to testing on multiple populations, this is not the case for methods of age assessment from the foot and ankle, for which only two original methods have been published (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002), one of which has been tested on a single occasion only (Hackman *et al.*, 2013). As a result of the omission of age estimation from the foot and ankle from testing, it is considered imperative that such an analysis is undertaken on both the Whitaker *et al.* (2002) and Hoerr *et al*. (1962) methods.

1.2 Development, growth and maturation of the long bones

To enable methods of skeletal age estimation to be developed, tested and applied in an appropriate manner, it is imperative that researchers and practitioners have a solid understanding of the structures and processes involved in skeletal development, growth and maturation.

1.2.1 Skeletal development and maturation

1.2.1.1 Skeletal ossification

Ossification may occur through either intramembranous or endochondral means. Intramembranous ossification can be further subdivided into dermal and perichondral ossification through which, diploic and cortical bone are formed respectively (Scheuer and Black, 2000; Mackie *et al.*, 2011). Although intramembranous ossification is largely restricted to the flat bones of the cranium, the lateral third of the clavicle and the blade of the scapula, endochondral ossification occurs throughout the skeleton and results in the formation of cancellous bone (Gardner, 1963; Ogden and Phillips, 1983; Ogata and Uhthoff, 1990; Scheuer and Black, 2000).

Endochondral ossification is a complex process that requires strict temporal and spatial regulation and results in the ossification of a cartilaginous model (Wallis, 1996; Mackie *et al.*, 2011). Following the secretion of angiogenic signalling molecules by hypertrophic chondrocytes, vascular invasion of a cartilaginous template or anlage occurs (Gerber and Ferrara, 1999; 2000; Fritsch *et al.*, 2001). This process, termed "silent angiogenesis" stimulates initial ossification through the invasion of osteoblasts and the formation of primary woven bone (Vortkamp *et al.*, 1996; Gerber and Ferrara, 2000). This process forms a bony collar in the centre of the cartilaginous anlage from which the remainder of the cartilaginous template will ossify.

The rate at which ossification progresses is both strictly controlled and site specific and can be altered by genetic or environmental influences (Stevens and Williams, 1999; Rivas and Shapiro, 2002). As the process is dependent on the rate of proliferation and differentiation of chondrocytes, it is hypothesised that cell signalling molecules such as parathyroid hormone related protein (PTHrP) and Indian hedgehog (Ihh) modulate the rate of chondrocyte maturation and thereby influence the rate of longitudinal bone growth and the timing of epiphyseal fusion (van der Eerden *et al.*, 2003). Some researchers have attempted to address the functions of the Ihh molecule and PTHrP in the regulation of endochondral ossification, including the possible existence of a negative feedback loop which

delays the differentiation of chondrocytes (Lanske *et al.*, 1996; Vortkamp *et al.*, 1996).

1.2.1.2 Skeletal maturation

Skeletal maturation is an extended process which requires the completion of normal development in over 300 separate centres of ossification. Categorised as either primary (1°) or secondary (2°), these sites of ossification form independently and where a secondary site of ossification is present, eventually fuse, leaving a single bone exhibiting adult morphology; however the age of onset and duration of epiphyseal fusion varies between bones (Nilsson and Baron, 2004). Secondary centres of ossification may be further classified as true epiphyses (sometimes referred to as pressure epiphyses); as apophyses, which may be defined as osseous projections that form as a result of muscular attachment and are therefore often termed "*traction epiphyses*" or as atavistic epiphyses which are considered a functional remnant of previous evolutionary forms (Parsons, 1904; Sullivan *et al.*, 1924; Barnett and Lewis, 1958). Although some 1° centres of ossification are associated with a solitary 2° centre, some bones, such as vertebrae, form from multiple primary centres of ossification (Scheuer and Black, 2000). Alternatively, some bones do not exhibit any secondary centres of ossification.

The appearance and progressive maturation of 2° centres of ossification (epiphyseal and apophyseal) is one facet of skeletal development that may be examined in the context of skeletal age estimation. The timing and pattern of epiphyseal development and fusion has been thoroughly documented in many anatomical regions (Greulich and Pyle, 1959; Hoerr *et al.*, 1962; Johnston and Jahina, 1965; Pyle and Hoerr, 1969; Garn *et al.*, 1974; Even *et al.*, 1998; Scheuer and Black, 2000; Cameriere *et al.*, 2006). It is not practical to discuss the development of all regions of the skeleton included in this study. As the foot has received comparably little attention in the literature, this region will be discussed as an example of skeletal development and maturation.

1.2.1.3 Ossification and maturation of the foot

The foot generally forms from 26 primary centres of ossification and at least 20 secondary centres of ossification, although some variation in the number of sites of ossification from which the primary and secondary centres of ossification of the foot develop has been reported (O' Rahilly, 1953; Roche and Sunderland, 1959; Venning, 1961; Garn *et al.*, 1966; Leonard, 1974). In addition, accessory ossicles, such as the "*navicular secundarium*" or "*os paracuneiform*", may appear (Bizzaro, 1921; Morrison, 1953; Powell, 1961; Case *et al.*, 1998; Offenbecker and Case, 2012). Although some accessory ossicles, such as the "o*s trigonum*", may fuse to neighbouring bones, some remain as isolated nodules resulting in the symptoms associated with *os trigonum* syndrome (Davies, 2004; Kose *et al.*, 2006; Glard *et al.*, 2009).

Initial chondrification of the foot begins at approximately week 7, followed by the commencement of ossification in the phalanges and metatarsals at approximately 9 weeks and the calcaneus at approximately the 13th week of intrauterine development (Bernhardt, 1988; Matthews, 1998; Fritsch *et al.*, 2001). Ossification of the remaining cartilaginous anlagen of the foot continues in the postnatal period, until fusion of the final epiphysis of the first metatarsal is completed between 15 and 18 years of age (Hoerr *et al.*, 1962).

Although the timing of appearance of many of the pedal epiphyses has been well documented, there are some exceptions, for example the proximal epiphysis of the fifth metatarsal. This secondary centre of ossification forms lateral to the proximal tuberosity at the point of insertion for the tendon of peroneus brevis muscle and is therefore considered to be a traction epiphysis or apophysis (Rogers, 1928; Matthews, 1998). Initial radiographic observations suggested that this centre of ossification did not appear in females until at least 12 years, while the youngest male in whom an epiphysis was observed was 14 years of age (Flecker, 1932). This was contested by a later study which suggested that the appearance and fusion of this epiphysis would occur between 8.5 years and 12.7 years in females and 10.8 and 15.3 years in males (Hoerr *et al.*, 1962). Based on dry bone observations, this epiphysis is said to commence ossification between 9 and 10

years of age in females and 12 years of age in males, with the process of fusion lasting approximately 24 months (Scheuer and Black, 2000).

1.2.1.4 Factors that may influence skeletal development and maturation

Numerous genetic and environmental factors may affect the rate at which skeletal ossification and maturation progresses (Garn *et al.*, 1963; Even *et al.*, 1998). It has been suggested that while the order of ossification of primary and secondary centres is genetically determined, the time at which they appear and develop may be influenced by extrinsic variables for example adequate nutritional intake (Hertzog *et al.*, 1969; Garn and McCreery, 1970; Garn *et al.*, 1973b; Cardoso, 2007). As a result of the many influences to which skeletal development and maturation may be exposed, any estimation of age must be accompanied by an acceptable range of variation (Workshop of European Anthropologists, 1980).

Consequently, to apply an appropriate approach or method to skeletal age estimation, an appreciation of these factors is required. The following sections will discuss the potential role of some of these factors in juvenile skeletal development.

Genetic influences on skeletal development and maturation

It has been suggested that male individuals exhibit a greater level of skeletal maturity during embryonic development than females (Garn *et al.*, 1974). It is generally accepted that postnatal skeletal development in female individuals is advanced compared with that observed in males of an equivalent chronological age (Lampl and Jeanty, 2003). The difference in timings of skeletal development observed in females and males may vary from a matter of weeks in infancy to a number of years in adolescents (Flory, 1935; Hansman and Maresh, 1961). This is particularly evident in the timing of the adolescent growth spurt and the attainment of peak height velocity (PHV), which are reported to occur between the ages of 12.5 years and 15.5 years in males and in females some two years previously (Tanner, 1981). The relative delay in the timing of PHV in male individuals compared with their female counterparts results in a growth phase of longer duration and consequently a greater final stature (Humphrey, 1998).

There is some evidence which suggests that the variation in the tempo of skeletal development observed between females and males may be linked to the X-

chromosome (Garn *et al.*, 1969; Hertzog *et al.*, 1969; Even *et al.*, 1998). Several studies have been undertaken to examine the influence of the X-chromosome on the developing bones of the hand-wrist and foot-ankle by examining correlations between familial pairs and triplets (Garn *et al.*, 1963; Garn *et al.*, 1969; Hertzog *et al.*, 1969; Garn and McCreery, 1970). These studies indicated that as sister-sister correlations exceeded those of any other pair, there may be a degree of influence from the X-chromosome (Garn and McCreery, 1970). This hypothesis is further supported by Even *et al.* (1998) who reported the observation of a maturational deficit between females of normal karyotype (XX) with either normal or small stature and those individuals with Turner's syndrome (XO). It was noted that skeletal development in those individuals affected by the disorder lagged behind individuals of normal karyotype. Given the Turner's syndrome chromosome 23 genotype of XO, the results of this study, in conjunction with those studies previously mentioned, suggest that the X-chromosome may moderate the sequence and tempo of ossification (Garn *et al.*, 1963; Garn *et al.*, 1969; Hertzog *et al.*, 1969; Garn and McCreery, 1970). Although the tempo of skeletal maturation is related to the sex of the individual, the pattern of ossification is believed to be constant between the sexes (Hoerr *et al.*, 1962; Pyle and Hoerr, 1969).

Ancestral origin

It has been recorded that the age of pubertal onset and the tempo of skeletal development may vary between populations of different ancestral origins (Rikhasor *et al.*, 1999). As many commonly applied approaches to skeletal age estimation were based on a population of a single ancestry group (e.g. Caucasoid), it is imperative that any potential variation in the accuracy of these methods between population groups is understood and where necessary, taken into account through the development of population-specific standards (Greulich and Pyle, 1959; Tanner *et al.*, 1962; Nelson *et al.*, 2000). This is of particular importance as a result of increasing immigration of undocumented individuals requires more medico-legal assessments of skeletal age to be undertaken (Schmeling *et al.*, 2001; Schmeling *et al.*, 2003).

Although variation in the timing of pubertal onset and the rate of skeletal and dental maturation has been noted between populations of different ancestral

origins (Datta Banik *et al.*, 1970; Garn *et al.*, 1973a; Rikhasor *et al.*, 1999; Olze *et al.*, 2004) there appears to be a general consensus that although the variation in skeletal maturation between different ancestral groups may be partially due to a genetic effect, it is largely due to differing levels of available resources including socioeconomic status, nutrition and health status (Greulich, 1957; Datta Banik *et al.*, 1970; Rikhasor *et al.*, 1999; Schmeling *et al.*, 2000).

Socioeconomic status

It is considered to be self-evident that individuals of lower socioeconomic status may exhibit delayed skeletal development compared with that observed in age-matched individuals from more economically prosperous backgrounds (Garn *et al.*, 1973b; Olze *et al.*, 2004). This is believed to be related to the reduction in the levels of available resources, such as adequate nutrition and healthcare, that may be accessed by individuals with a lower income level or within a less affluent population or geographical area (Adamson *et al.*, 2003). The discrepancy in skeletal maturation between individuals of higher and lower socioeconomic backgrounds has been highlighted as a potential explanation for errors in estimations of skeletal age between populations (Greulich and Pyle, 1959; Groell *et al.*, 1999; Nelson *et al.*, 2000; Schmeling *et al.*, 2003).

The effects of socioeconomic status on skeletal growth and maturation begin during prenatal development as a result of the health and nutritional status of the mother (Bradley and Corwyn, 2002). Children of low-income families may be more likely to experience greater birth complications including premature birth and a lower birth weight than those of higher income families (Crooks, 1995; Poulton *et al.*, 2002). Low birth weight has in turn been associated with early onset menarche and reduced final stature in females and low bone mineral density in adult individuals (Paz *et al.*, 1993; Ibáñez *et al.*, 2000; Hovi *et al.*, 2009).

Factors related to low socioeconomic status continue to influence skeletal development throughout infancy and into childhood. It has been suggested that there may be a critical period in juvenile development during which the skeleton is most susceptible to influence from factors associated with the socioeconomic

status of the individual, their family, population or geographical area (Greulich, 1957; Cole and Cole, 1992).

Nutritional and health status

There is a general consensus within the literature that malnutrition may exert an effect on the tempo of ossification in the human skeleton (Dreizen, 1958; Frisancho *et al.*, 1970; He and Karlberg, 2001). While the term malnutrition may equally be applied to individuals of low and high body mass index (BMI), it is known that individuals who are severely underweight exhibit delayed skeletal maturation compared to those with a healthy BMI (Lacey *et al.*, 1979; Denzer, 2007). This may be attributable to numerous factors including hormonal imbalance, particularly in relation to oestrogen production and a low body mass, including a reduction in skeletal muscle mass and strength. Additionally, , Although some aspects of growth and skeletal maturation in individuals with childhood obesity are not yet fully understood, studies have shown that individuals who exhibit a high BMI during skeletal development mature in advance of those with a normal body mass index (Vignolo *et al.*, 1988; Russell *et al.*, 2001; Denzer, 2007).

Any form of disturbance in the nutritional balance of a developing individual has the potential to place the body into a state of physiological stress due to an imbalance in the nutrients required for normal metabolic activity. This physiological stress can result in a temporary arrest in the growth of long bones (Nowak and Piontek, 2002). This may result in the appearance of transverse radio-opaque lines known as Harris Lines, which form during the period of recovery following a growth disturbance (Harris, 1931; Nowak and Piontek, 2002; Papageorgopoulou *et al.*, 2011).

1.2.2 Epiphyseal fusion and the cessation of longitudinal growth

The process of skeletal age estimation is based on the initial ossification and progressive growth and development of bone until adult morphology is attained. To understand this process, it is first necessary to be cognisant of the underlying structures and their roles in skeletal growth. In terms of long bone growth, this includes an awareness of the epiphyseal growth plate and its component parts.

1.2.2.1 Growth plate histology

The human epiphyseal growth plate is formed from hyaline cartilage and is located at one, or both, of the proximal and distal ends of long or short bones (van der Eerden *et al.*, 2003; Sadler, 2010; Forcinito *et al.*, 2011). In simple terms, the human growth plate may be considered to comprise three regions or zones; the resting zone (also known as the germinal zone), the zone of proliferation, and the hypertrophic zone (Figure 1.1), all of which refer to different stages of chondrocyte morphology during the cell cycle (Brighton, 1978; Weise *et al.*, 2001; Abad *et al.*, 2002; Nilsson *et al.*, 2005; Burdan *et al.*, 2009; Emons *et al.*, 2009).

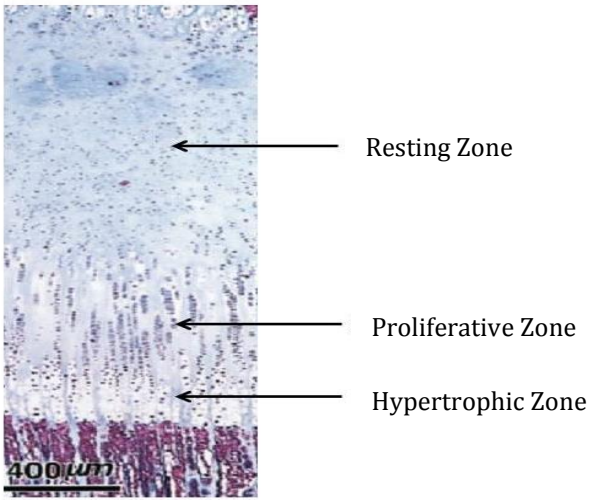

Figure 1.1: Zones of the Mammalian Growth Plate; Adapted from Abad *et al.* (2002)

There appears to be a degree of discord within the literature however concerning the number of zones contained within the human epiphyseal growth plate and the nomenclature used to describe them (Brighton, 1978; Scheuer and Black, 2000; Burdan *et al.*, 2009). During the life of a chondrocyte, it passes through sequential stages of maturation, proliferation, differentiation and apoptosis after which it is succeeded by the formation of new bone (Stevens and Williams, 1999).

The Resting Zone

The resting zone is the region of the growth plate located farthest from the area of new bone formation. The resting zone is poorly organised as chondrocytes are scattered irregularly amongst the cartilaginous matrix (Abad *et al.*, 2002). Each zone of the growth plate plays a crucial role in longitudinal bone growth however the function of the resting zone is relatively poorly understood (Abad *et al.*, 2002).

As its name suggests, the resting zone was originally thought to be inert (Kember and Sissons, 1976) and contain only resting chondrocytes (Ballock and O' Keefe, 2003). Recent research however has suggested a number of potential roles of this zone in relation to longitudinal bone growth which are summarised below (Abad *et al.*, 2002; Schrier *et al.*, 2006).

1. The resting zone contains stem-like progenitor cells from which the proliferative chondrocytes are derived. It has been suggested by several authors that the stem-like cells within the resting zone have a finite proliferative capacity which, when exhausted, leads to cessation of longitudinal growth and closure of the epiphyseal growth plate (Kember and Walker, 1971; Ballock and O' Keefe, 2003; van der Eerden *et al.*, 2003; Nilsson and Baron, 2005; Nilsson *et al.*, 2005; Schrier *et al.*, 2006).
2. The resting zone secretes a compound which directs the alignment of proliferative and hypertrophic chondrocytes and inhibits differentiation of proliferative chondrocytes via chemotaxis (Abad *et al.*, 2002; Fischer *et al.*, 2010).

The Proliferative Zone

Within the proliferative zone chondrocytes undergo a process of binary fission (Abad *et al.*, 2002). The rate of proliferation within this zone is rapid, synthesising cartilaginous matrix, expanding the growth plate and facilitating longitudinal growth (Buckwalter *et al.*, 1985). Cells within the proliferative zone are arranged in columns which lie parallel to the long axis of the bone, thereby facilitating unidirectional bone growth (Hunziker and Schenk, 1989; Weise *et al.*, 2001).

It is believed that a chemotactic process regulates the alignment of these cells (Abad *et al.*, 2002), although the precise mechanism by which this occurs is not yet understood (van der Eerden *et al.*, 2003). Several authors have suggested that stimulation of the secretion of the Ihh molecule by prehypertrophic chondrocytes controls the differentiation of proliferative chondrocytes, while exposure to PTHrP restricts the differentiation of proliferative chondrocytes into hypertrophic cells (St-Jacques *et al.*, 1999; Kronenberg, 2003; Fischer *et al.*, 2010).

The Hypertrophic Zone

The hypertrophic zone is most closely associated with the region of new bone formation (Abad *et al.*, 2002). As in the proliferative zone, the cells within the hypertrophic zone lie in a columnar arrangement, typically including fifteen to seventeen chondrocytes running parallel to the long axis of the bone (Cowell *et al.*, 1987; Stevens and Williams, 1999). Although generally considered to be a single region of the growth plate, the hypertrophic zone can be separated into an upper hypertrophic (maturation zone) and a lower hypertrophic (degenerative zone) in relation to their position relative to the proliferative zone (Cowell *et al.*, 1987; Wallis, 1996).

Within the hypertrophic zone, chondrocytes alter in both size and shape becoming larger and more spherical than in the resting or proliferative zones (Buckwalter *et al.*, 1986). It is suggested by Hunziker *et al.* (1987) that hypertrophic cell volume can increase by a factor of 10 and cellular height can increase by a factor of 4 relative to prehypertrophic chondrocytes. This is supported by Stevens and Williams (1999) who state that hypertrophic cell height may be up to five times greater than that of a proliferative chondrocyte.

Cells of the hypertrophic zone are functionally distinct from those of the proliferative zone and produce matrix proteins that are not secreted by the younger chondrocytes. These compounds are released into the extracellular matrix (ECM) which surrounds the hypertrophic cells and stimulate angiogenesis prior to the commencement of endochondral ossification (Gerber and Ferrara, 1999; Schinke, 1999). As cellular proliferation continues and hypertrophic chondrocytes undergo programmed cell death or apoptosis, the underlying processes which facilitate longitudinal bone growth, are initiated. It has been suggested that following chondrocyte hypertrophy, vascular invasion occurs resulting in induction of mineralisation within the cartilaginous matrix (Buckwalter *et al.*, 1986).

1.2.2.2 Chondrocytes, the growth plate and epiphyseal fusion

As a result of the speed with which epiphyseal fusion occurs, few studies have been conducted on tissue from healthy individuals, although some have been

undertaken using slipped capital femoral epiphyses (Adamczyk *et al.*, 2005; Emons *et al.*, 2009). Although murine models have been used in some studies, the similarities between the process of epiphyseal fusion in animals and humans have been disputed, particularly in rodents as epiphyseal fusion does not appear to be triggered by sexual maturation (Nilsson *et al.*, 2005; Emons *et al.*, 2009; Forcinito *et al.*, 2011).

It has been proposed that the chondrocytes responsible for linear bone growth have a finite proliferative capacity, which, when exhausted, results in a cessation of growth (van der Eerden *et al.*, 2003; Nilsson and Baron, 2004; Schrier *et al.*, 2006). The reduction in proliferation rate observed in the chondrocytes is accompanied by a reduction in the height of the growth plate and eventually results in the cessation of longitudinal growth and epiphyseal fusion (Weise *et al.*, 2001; Rivas and Shapiro, 2002; Nilsson and Baron, 2004; Schrier *et al.*, 2006; Emons *et al.*, 2009). It has been suggested that control of the proliferation rate of hypertrophic chondrocytes is intrinsic to the growth plate (Ballock and O' Keefe, 2003). This hypothesis is supported by the results obtained by Stevens *et al*. (1999) who found that following transplantation, the proliferation rate of the transplanted chondrocytes was dependent on the age of the donor animal rather than that of the recipient.

Following the cessation of longitudinal bone growth, the primary and secondary centres of ossification fuse, resulting in a single solid structure (Nilsson and Baron, 2004). Although the overall pattern of epiphyseal fusion is relatively constant between individuals, the time at which the process starts, and completes, varies and may be influenced by numerous genetic and environmental factors. Studies such as those by Schaefer (2008); and Schaefer and Black (2005) have shown that differences may exist in the timing of epiphyseal fusion between different populations. It has been suggested that the discrepancies in the timing of epiphyseal fusion observed between populations may be due to variability of socioeconomic status and the associated inequalities in the acquisition of resources, such as adequate nutrition and healthcare, rather than being solely related to genetic variation *per se* between populations or ethnicities (Todd, 1931; Garn *et al.*, 1973b; Schmeling *et al.*, 2000; Schmeling *et al.*, 2006).

1.2.2.3 Factors that may influence the timing of epiphyseal fusion

Perhaps the greatest determinant of the timing of epiphyseal fusion is the hormonal fluctuation that is associated with the onset of puberty, which itself is determined by both genetic and environmental factors (Gluckman and Hanson, 2006a; Toppari and Juul, 2010). It has been suggested however that the degree of influence that environmental factors may have on the timing of the commencement of puberty may be genetically determined (Pinyerd and Zipf, 2005; Gluckman and Hanson, 2006a; 2006b; Gajdos *et al.*, 2010; Toppari and Juul, 2010). It has been suggested that numerous factors may exert an influence on the timing of onset and tempo of puberty including pre- and postnatal nutrition, body mass, gonadal dysfunction and accidental chemical and heavy metal exposure (Pinyerd and Zipf, 2005; Toppari and Juul, 2010). A thorough review of the environmental influences on the onset of puberty can be found in Toppari and Juul (2010).

In both females and males, the onset of puberty is accompanied by a rise in the levels of circulating hormones including oestrogen, which, through the presence of receptors in the growth plate and its interaction with growth hormone, stimulates the process of epiphyseal fusion (Juul, 2001; van der Eerden *et al.*, 2003; Nilsson and Baron, 2004; Perry *et al.*, 2008). The occurrence of delayed puberty, growth spurt and the concomitant absence of increased levels of circulating hormones may result in late epiphyseal fusion (Weise *et al.*, 2001; Perry *et al.*, 2008). Conversely, factors that encourage the precocious onset of puberty may result in advanced skeletal maturation and epiphyseal fusion (Nilsson *et al.*, 2005; Perry *et al.*, 2008). A more in depth discussion of the role of oestrogens in bone formation and remodelling can be found in section 1.4.3.1.

1.3 Aetiology and interpretation of epiphyseal scars

Following epiphyseal fusion, a thin radio-opaque line termed the "*epiphyseal scar*", may be observed in the location of the former growth plate. Unlike other forms of transverse radio-opaque lines, such as Harris lines, the aetiology of the epiphyseal scar has received little attention within the literature (Harris, 1931).

Consequently, it is necessary to consider the aetiology of Harris lines as a potential explanation of the origin of epiphyseal scars.

During a temporary period of growth inhibition, such as may occur during a period of physiological stress, a deceleration of chondrocyte proliferation rate occurs, resulting in structural changes within the growth plate (Nilsson and Baron, 2004). This includes a reduction in the overall height of the proliferative zone of the growth plate, a decline in the size of hypertrophic cells and a reduction in the density of the hypertrophic zone (Ballock and O' Keefe, 2003). As chondrocyte proliferation slows, osteoblasts cannot progress and consequently settle on the epiphyseal growth plate, resulting in an increased deposition of bone and the appearance of a transverse radio-opaque line. The mechanisms behind the deceleration of growth rate prior to transient growth inhibition during childhood and those observed leading up the final cessation of growth seem to involve the same reduction in chondrocyte proliferation rate and growth plate height and could therefore represent a possible aetiology of the epiphyseal scar (Park, 1954; Nilsson and Baron, 2005; Cunningham and Stephen, 2010).

1.3.1 Persistence of epiphyseal scars in adults

The potential persistence of epiphyseal scars in adult individuals was first noted by Cope (1920), whose findings were supported by those of Paterson (1929) who noted that "*An exception is made of the line like mark which sometimes persists into adult life – the so-called epiphyseal scar*". A year later, Todd (1930) examined the timing of epiphyseal union in living individuals. Although this study examined the developmental progress of the juvenile hand skeleton, the potential persistence of the epiphyseal scar in skeletally mature individuals was acknowledged:

> "*The white line of the roentgenogram persists as a fine scar for some months. It may remain throughout life as it often does in the upper tibia, or it may disappear after approximately 6 months as it always does in the lower ulna and somewhat less often in the lower radius. We have defined this stage as recent union*".

This was echoed fifty years later by the Workshop of European Anthropologists (1980) who, in their recommendations for age determination of adolescents, stated:

> *"The epiphyseal lines are noticeable for approximately one to two years after ossification. These point to the transition into the adult age"*

Consequently, the obliteration of this feature has also been included as the final maturity indicator in a number of methods of radiographic juvenile age estimation (Whitaker *et al.*, 2002; Schmidt *et al.*, 2008; Baumann *et al.*, 2009).

1.3.2 The epiphyseal scar in skeletal age estimation

As the demarcation of the completion of epiphyseal fusion, the presence of an epiphyseal scar is intrinsically linked with skeletal maturation and therefore has been recognised as a feature in skeletal age assessment (Hoerr *et al.*, 1962; Pyle and Hoerr, 1969; Workshop of European Anthropologists, 1980; Webb and Suchey, 1985; Kreitner *et al.*, 1998; Whitaker *et al.*, 2002; Schmidt *et al.*, 2007b; Schmidt *et al.*, 2008; Baumann *et al.*, 2009; Garamendi *et al.*, 2011). While the relationship between the initial formation of this structure and the stage of skeletal development of the individual is not in doubt, the affiliation between skeletal age and the obliteration of the epiphyseal scar is a matter of contention. In some skeletal regions, such as the medial clavicle, it is widely accepted that the epiphyseal scar will obliterate soon after the completion of fusion (Webb and Suchey, 1985; Kreitner *et al.*, 1998; Schulz *et al.*, 2005; Kellinghaus *et al.*, 2010; Garamendi *et al.*, 2011). In other skeletal areas, the potential persistence of epiphyseal scars in adult individuals is a matter of contention as some authors consider that the feature may be retained throughout adulthood (Greulich and Pyle, 1959; Hoerr *et al.*, 1962; Hall and Rosser, 1963; O'Connor *et al.*, 2008); while others employ the obliteration of the epiphyseal scar as a criterion in skeletal age assessment (Thiemann and Nitz, 1991; Whitaker *et al.*, 2002; Schmidt *et al.*, 2008; Baumann *et al.*, 2009).

As the potential persistence of an epiphyseal scar varies between skeletal elements, it is necessary to consider the influence that this feature may exert on estimations of chronological age in multiple areas. A brief summary of published

approaches to skeletal age assessment, including the role of the epiphyseal scar, is presented in this section.

1.3.2.1 Medial clavicle

The clavicle is the first bone in the human skeleton to commence ossification, which occurs at approximately week 6 of intrauterine development. Despite preceding the remainder of the skeleton, the clavicle is also one of the last bones to complete epiphyseal fusion, which is likely to occur in the region of the medial epiphysis within the third decade of life (Flecker, 1932; Black and Scheuer, 1996). As a result of its prolonged development, the clavicle is of particular interest in skeletal age estimation, particularly in the context of living individuals, where a practitioner may be required to establish whether an individual has reached the age of 18 years (Walker and Lovejoy, 1985; Kreitner *et al.*, 1998; Schmeling *et al.*, 2003; Schmeling *et al.*, 2005b; Schulz *et al.*, 2005; Schulze *et al.*, 2006; Schmidt *et al.*, 2007b; Schmeling *et al.*, 2008; Garamendi *et al.*, 2011; Schulz *et al.*, 2013).

Due to the frequency with which examination of the medial extremity of the clavicle is undertaken in skeletal age estimation in living individuals, this anatomical region has received significant attention from multiple research groups using a variety of modalities of clinical imaging including plain film radiography (Walker and Lovejoy, 1985; Schmeling *et al.*, 2004; Garamendi *et al.*, 2011), computed tomography (Kreitner *et al.*, 1998; Schulze *et al.*, 2006; Kellinghaus *et al.*, 2010), magnetic resonance imaging (Schmidt *et al.*, 2007b; Hillewig *et al.*, 2011; Tangmose *et al.*, 2013), and ultrasonography (Quirmbach *et al.*, 2009; Gonsior *et al.*, 2013; Schulz *et al.*, 2013). To quantify the level of epiphyseal fusion observed, many studies utilise a scoring system that frequently includes the obliteration of the epiphyseal scar as the final maturity criterion (Schmeling *et al.*, 2004; Schulz *et al.*, 2005; Schulz *et al.*, 2008b; Kellinghaus *et al.*, 2010; Hillewig *et al.*, 2011). While no studies have explicitly undertaken an examination of the epiphyseal scar in this region, there appears to be a consensus among researchers that the epiphyseal scar of the medial clavicle will obliterate soon after the completion of epiphyseal fusion.

1.3.2.2 Proximal humerus

Examination of the shoulder joint, and in particular, the proximal humerus in skeletal age estimation has, in juvenile individuals, largely been restricted to the pattern and timing of epiphyseal coalescence and fusion of the humeral head (Scheuer and Black, 2000); and in skeletally mature individuals, to the expansion of the humeral medullary cavity and loss of trabecular bone within the humeral head associated with senescent change (Acsadi and Nemeskeri, 1970).

Although no studies have specifically examined the epiphyseal scar in the proximal humerus, the potential persistence of the feature was noted in a study by Hall and Rosser (1963) in their examination of age-related osteoporotic changes. Despite loss of the trabecular structure of the greater tuberosity and expansion of the medullary cavity to the approximate region of the epiphyseal scar, a persistent epiphyseal scar was observed separating the diaphyseal and epiphyseal regions in all samples examined in this study (Hall and Rosser, 1963). This finding was supported by the later work of MacLaughlin (1987), who noted that "*evidence of the epiphyseal plate persisted even where there was extensive resorption both distal and proximal to it*". This therefore suggests that an epiphyseal scar may commonly persist in the proximal humerus in skeletally mature individuals.

1.3.2.3 Distal humerus, proximal radius and proximal ulna

Skeletal maturation of the elbow region involves the appearance and fusion of six secondary centres of ossification including those for the proximal radius and ulna; and those of the trochlea, capitulum and medial and lateral epicondyles of the distal humerus (Scheuer and Black, 2000). Approaches to skeletal age estimation based on this region include those by Sauvegrain (Diméglio *et al.*, 2005; Canavese *et al.*, 2008) and Brodeur *et al*. (1981). Although epiphyseal scars are not referred to in the test of the Sauvegrain method (Diméglio *et al.*, 2005), the radiographic atlas of Brodeur *et al*. (1981) refers to the absorption of "physeal lines" which accompanies the completion of skeletal maturation in the elbow region. The absence of reference to the feature by Sauvegrain and the affirmation of obliteration by Brodeur *et al.* (1981) suggests that complete obliteration of epiphyseal scars is likely to occur in all skeletal elements in this anatomical region.

1.3.2.4 Distal radius, distal ulna, metacarpals and short bones of the hand

The epiphyseal scar of the distal radius has been referred to in numerous age estimation methods and studies throughout the past century including the major atlases produced by the research groups of Greulich and Pyle (1950; 1959) and Tanner *et al.* (1962; 1975; 2001). Although a recognised feature of skeletal development, the fate of the epiphyseal scar, of the distal radius in particular, is a matter of debate within the literature where it is recognised by some authors as a potentially persistent feature while others refer either to the disappearance of the feature or make no reference to its presence (Todd, 1937; Greulich and Pyle, 1950; 1959; Thiemann and Nitz, 1991; Gilsanz and Ratib, 2005). This is potentially due to the absence of specific data relating to the persistence of the feature in skeletally mature individuals. This problem was partially addressed by the work of Baumann *et al.* (2009) by extending the age range of the individuals included in their study to 30 years of age. Although this study included the obliteration of the epiphyseal scar as the final maturity stage, it was acknowledged that the feature may remain visible in the distal radius in some adult individuals (Baumann *et al.*, 2009).

As one of the triumvirate of methods recommended for age estimation in the living by the German Working Group on Forensic Age Diagnostics (AGFAD), the hand and wrist represents the most widely used area for skeletal age estimation in both living and deceased individuals (Schmidt *et al.*, 2008; Hackman and Black, 2013b) (Hackman, 2012). The importance of the validation of the role of the epiphyseal scar within age estimation from this region cannot be underestimated, particularly as many assessments of skeletal age undertaken on living individuals have the aim of assessing whether the individual has reached 18 years of age (Schmeling *et al.*, 2003; Schmeling *et al.*, 2007; Schmeling *et al.*, 2008; Baumann *et al.*, 2009).

1.3.2.5 Innominate and proximal femur

Skeletal development and maturation of the innominate and proximal femur includes the appearance and fusion of multiple secondary centres of ossification including those for the head, greater trochanter and lesser trochanter of the femur; and the traction epiphyses of the ischium and ilium, in addition to the coalescence

of the innominate from its component elements, the ischium, ilium and pubis (Scheuer and Black, 2000). As a result of the potential harm caused by exposure to ionising radiation, this anatomical region has been largely excluded from radiographic approaches to skeletal age estimation in juveniles (Dewey *et al.*, 2005). Several studies have, however, attempted to associate the ossification and fusion of the iliac crest apophysis with skeletal age utilising clinical radiographic samples (Thaler *et al.*, 2008; Modi *et al.*, 2009; Schmidt *et al.*, 2011; Wittschieber *et al.*, 2013a; Wittschieber *et al.*, 2013b; Wittschieber *et al.*, 2013c). Although the observation of an apophyseal scar in this region was not an established objective of the evaluation of ossification of the iliac crest apophysis conducted by Wittscheiber *et al.* (2013b), it was noted that within the sample of radiographs examined, no apophyseal scars were observed in the region of the iliac crest.

Within the anatomical region of the hip, the femoral capital epiphysis and those of the greater and lesser trochanter remain a source of information relating to chronological age. Although data have been compiled relating to the development of the proximal femur and skeletal age estimation based on the gross inspection of remains, the region has been largely omitted from radiographic methods of juvenile age assessment as a result of the potential health and safety implications of exposure to ionising radiation (Scheuer and Black, 2000; Gogos *et al.*, 2003). Reference has however been made to the femoral capital epiphyseal scar which has been reported to become progressively less distinct with advancing age (Parsons, 1904; Garden, 1961). No evidence has been located within the literature to support this statement. Attention has also been paid to this feature in relation to the incidence of femoral neck fractures (Tamai *et al.*, 1983). It was noted in this study that epiphyseal scars were observed in individuals up to approximately 90 years of age. Based on their findings, Tamai *et al.* (1983) explicitly concur with previous findings regarding the persistence of the epiphyseal scar in adult individuals (Klenerman and Marcuson, 1970).

1.3.2.6 Distal femur and proximal tibia

Although few in number, the methods of age estimation from the knee present a variety of opinions regarding the persistence of the epiphyseal scar in the proximal

tibia and distal femur (Pyle and Hoerr, 1969; O'Connor *et al.*, 2008; Cameriere *et al.*, 2012; Kausar and Varghese, 2012; O'Connor *et al.*, 2012).

The apparent uncertainty surrounding the epiphyseal scar may be summarised in the examination of a study by Cameriere *et al.* (2012) in which a 3-stage system was developed and applied to assess the skeletal age of individuals from radiographic images of the knee. Although the application of staging systems is an accepted and widely applied method in age estimation studies (Whitaker *et al.*, 2002; Schulz *et al.*, 2005; O'Connor *et al.*, 2008), the sensitivity of the method by Cameriere *et al*. (2012) may be low due to an insufficient number of stages and the ambiguous descriptions of the criterion with which each stage is associated (Whitaker *et al.*, 2002). The criterion of the final stage of maturity applied within this study states "*epiphysis is fully ossified and epiphyseal scar is not visible*". Several problems are encountered in the examination of this method, including the use of the term "ossification" within the stage criteria and the omission of reference to epiphyseal fusion. In addition, the exemplar image corresponding to the final maturity stage clearly shows an epiphyseal scar (Figure 1.2).

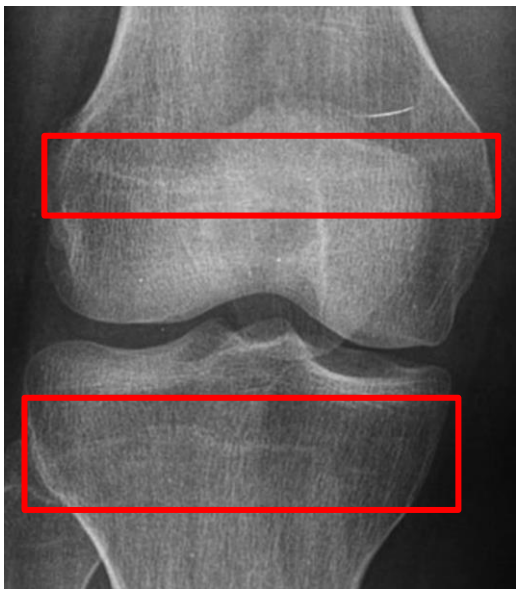

Figure 1.2: Exemplar image of Cameriere's stage 3 which shows the presence of an epiphyseal scar in the distal femur and proximal tibia (boxed). Adapted from Cameriere *et al.* (2012)

It is considered by O'Connor *et al.* (2008) that following completed fusion, a thin epiphyseal scar may remain in some cases. Although no literature is cited to reinforce this statement, it is supported by the maturity criteria found within the

radiographic standard of reference for the growing knee (Pyle and Hoerr, 1969). Examination of the literature relating to the epiphyseal scar in the assessment of age in the knee and the degree of discord observed therein supports the requirement for specific examination of epiphyseal scars in the distal femur and proximal tibia to be undertaken, particularly if the aim of the method is to assess whether an individual has attained the age of 18 years (Pyle and Hoerr, 1969; O'Connor *et al.*, 2008; Cameriere *et al.*, 2012; Kanchan and Krishan, 2012; O'Connor *et al.*, 2012).

1.3.2.7 Distal tibia, distal fibula, metatarsals and short bones of the foot

Although the distal portion of the leg and the foot tend to be protected from loss or damage by footwear, and as a result may be recovered from disruptive events such as perimortem fragmentation or postmortem disarticulation, little attention has been paid to this region in the context of skeletal age estimation (Işcan and McCabe, 1995; Haglund and Sorg, 1996). As a result, only two methods of age estimation based on this anatomical area have been produced in the past 51 years, and therefore the discussion of the inclusion of the epiphyseal scar in relation to age estimation from the foot and ankle is limited (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002).

The first method of skeletal assessment of the foot and ankle was produced by Hoerr *et al.* (1962) in the format of a radiographic atlas, similar to those produced for the knee (Pyle and Hoerr, 1969) and hand and wrist (Greulich and Pyle, 1950; 1959). Within this volume, repeated reference is made to the possible persistence of the epiphyseal scar or "*terminal line*" in the distal tibia and fibula, metatarsals and all phalangeal rows. The inclusion of the epiphyseal scar within this radiographic atlas suggests that it was commonly observed in the images examined during the longitudinal study on which it was based. As no reference is made to the observation of an epiphyseal scar in the calcaneus, it is inferred that no such finding occurred.

In contrast to the work by Hoerr *et al.* (1962), the Whitaker *et al*. (2002) method for estimating age from the bones of the foot includes the obliteration of the epiphyseal scar as a criterion according to which maturity scores were assigned.

This therefore suggests that the authors did not consider the epiphyseal scar to be a persistent feature in the bones examined by their method. No explanation was given regarding the reason for the choice of maturity criteria used in this study. Although the method presented by Whitaker *et al*. (2002) was only the second published method of age estimation from this anatomical region, it appears that attention was not paid to the work of Hoerr *et al*. (1962) in the development of the method. As a result, ambiguity regarding the persistence or obliteration of epiphyseal scars in the foot and ankle of adult individuals has been introduced and it is therefore imperative that this is addressed through further research.

Recently, new evidence for the possible persistence of some epiphyseal scars has been reported (Weiss *et al.*, 2012). This study showed that persistent epiphyseal scars were encountered in 38% of individuals of known chronological age between 17 and 88 years of age. These findings suggest that the epiphyseal scar of the first metatarsal may persist in skeletally mature individuals, and therefore the suggestion by Hoerr *et al*. (1962) that the epiphyseal scar or terminal line may persist. Weiss *et al*. (2012) also noted the potential implications of persistent epiphyseal scars for skeletal age assessment from the foot in the forensic context and advised caution in the interpretation of the epiphyseal scar as an indicator of recent epiphyseal fusion.

1.4 Bone remodelling in the adult skeleton

The reported obliteration of epiphyseal scars may only arise through alteration to the underlying cancellous structure, which occurs through the process of bone remodelling. The demands placed on the skeleton through the application of mechanical loads and metabolic processes require constant alterations to be made to the bone in order to maintain structural competency and normal metabolic activity (Martin and Sims, 2005; Li *et al.*, 2006; Sims and Gooi, 2008; Henriksen *et al.*, 2009). As bone is a dynamic structure, these processes are continuous and facilitate skeletal adaptation to meet physical and physiological requirements (Hill, 1998; Väänänen *et al.*, 2000; Boyle *et al.*, 2003; Väänänen and Laitala-Leinonen, 2008). As alteration to the cancellous structure may result in obliteration of the epiphyseal scar, it is necessary to consider the process and function of bone remodelling and those factors that may exert an influence on the rate of bone

remodelling throughout the skeleton, or within a localised area such as the epiphyseal scar.

1.4.1 Process and function of bone remodelling

Bone remodelling is a complex, cyclical process which, through the cooperative actions of osteoclasts and osteoblasts, results in the removal of unnecessary or damaged bone and its replacement with newly deposited osteoid which, once mineralised, forms new bone (Suda *et al.*, 1997; Hill, 1998; Väänänen *et al.*, 2000; Boyle *et al.*, 2003; Martin and Sims, 2005; Väänänen, 2005; Li *et al.*, 2006; Sims and Gooi, 2008; Väänänen and Laitala-Leinonen, 2008; Henriksen *et al.*, 2009). In a healthy individual and under 'normal' conditions, the processes of bone resorption and formation are coupled and as a result, the quantity of bone resorbed is largely equivalent to that deposited, thereby maintaining the quantity and quality of bone present (Hill, 1998; Väänänen, 2005; Karsdal *et al.*, 2008; Henriksen *et al.*, 2009). Although linked, the processes of bone resorption and formation occur at different rates, with the resorption phase being significantly shorter than the formation phase and consequently a greater proportion of the skeleton will be undergoing resorption than formation at any one time (Kimble, 1997). Due to the high surface area of cancellous bone compared with cortical bone, a larger proportion of this bone will be undergoing remodelling at any one time, resulting in a greater loss of cancellous bone than cortical bone (Vogel *et al.*, 1997).

The process of bone remodelling requires the completion of a number of phases, without which, the requisite cell types and signalling molecules are not produced. The initial stage of the remodelling cycle includes the production of osteoclasts from haematopoietic progenitor cells (Suda *et al.*, 1997; Väänänen *et al.*, 2000; Li *et al.*, 2006). This process, termed osteoclastogenesis, is believed to be mediated through the production of signalling molecules by osteoblasts (Henriksen *et al.*, 2009). Through their interaction with the receptor activator of nuclear factor K B (RANK) receptor on the surface of the haematopoietic progenitor cells, the signalling molecules of Receptor Activator of Nuclear Factor K B Ligand (RANKL) and Macrophage-Colony Stimulating Factor (M-CSF) secreted by the osteoblasts are important in the production of osteoclasts (Väänänen *et al.*, 2000; Boyle *et al.*, 2003; Martin and Sims, 2005; Henriksen *et al.*, 2009). This process however is

mediated by the secretion of osteoprotegerin (OPG) by osteoblasts which binds to the RANKL molecule, thus preventing the RANK-RANKL interaction that stimulates osteoclastogenesis (Väänänen *et al.*, 2000; Boyle *et al.*, 2003).

Although osteoclasts originate from the same lineage as other macrophages, they possess characteristics that make them well suited to their unique function as the only cell type capable of dissolving mineralised bone tissue (Väänänen *et al.*, 2000; Väänänen and Laitala-Leinonen, 2008). Once the cell has migrated to the site at which remodelling is to take place, polarisation of the cell occurs as a result of the influence of vitronectin receptor molecules which bind to a tripeptide arginine-glycine-aspartic acid (RGD) recognition site (Hill, 1998). This process results in the osteoclast dividing into clear and ruffled regions which is followed by the formation of four membranous domains: Ruffled Border; Functional Secretory Domain; Basolateral Domain; and the Sealing Zone, through which the osteoclast attaches to the bone surface beneath the cell (Väänänen *et al.*, 2000).

Once attached, the osteoclast begins to dissolve the underlying bone through the secretion of concentrated hydrochloric acid (HCl) and the protease Cathepsin K (Matsuo and Irie, 2008; Väänänen and Laitala-Leinonen, 2008). This produces a matrix of dissolved calcium and phosphate which, through cellular transduction, is removed from the resorbed lacuna and released through the functional secretory domain into the extracellular space (Väänänen *et al.*, 2000). The mode by which bone resorption is ultimately halted is not yet fully understood, however it is suggested by Li *et al.* (2006) that a chemotactic response to high Ca^{2+} levels in the area surrounding the site of osteoclastic activity results in withdrawal of the cell from the region and therefore the cessation of bone resorption. This is followed by the apoptosis of osteoclasts, which, it is suggested by Suda *et al*. (1997), is susceptible to hormonal influence as a result of the interaction between oestrogen and osteoblasts and the associated increase in production of Transforming Growth Factor–β (TGF–β) which stimulates osteoclast apoptosis.

According to Matsuo and Irie (2008), the area of remodelling then enters a period of transition, during which the retreating osteoclasts stimulate the differentiation of osteoblastic precursors to produce mature osteoblast cells. This phase is

reported to include the “Reversal Phase”, during which remaining Type I collagen strands are removed from the resorption lacunae through the action of matrix metalloproteinases (MMPs) and the newly resorbed surface is conditioned for bone formation (Everts *et al.*, 2002; Sims and Gooi, 2008; Henriksen *et al.*, 2009). It has been suggested that this process is undertaken by mononuclear bone lining cells of osteoblastic lineage and is required prior to the deposition of osteoid matrix and the formation of new bone (Everts *et al.*, 2002).

Following the transition or reversal phase, the period of bone formation begins (Matsuo and Irie, 2008; Sims and Gooi, 2008; Henriksen *et al.*, 2009). This process may be divided into three events which constitute the recruitment of osteoblast precursor cells, the proliferation of osteoblast precursor cells and finally the differentiation of these cells into osteoblasts (Hill, 1998). Through the deposition of osteoid matrix by osteoblasts, the bone removed during the resorption phase is replaced. This process continues until the resorbed cavity has been filled, however the action by which the osteoblasts detect the quantity of bone required is not fully understood (Hill, 1998; Sims and Gooi, 2008). It has been suggested that osteoblasts preferentially deposit osteoid in areas where the underlying osseous topography has been altered and detect the size and shape of the defect (Hill, 1998; Sims and Gooi, 2008). In their study of bone lining cells, Everts *et al*. (2002) suggest that in addition to removing remaining vestiges of collagen from the resorbed lacuna, the bone lining cells deposit a thin layer of collagen on which new bone is deposited by osteoblasts.

Although it may seem counterintuitive, osteoclasts are reported to play a vital role in the formation of new bone (Sims and Gooi, 2008; Henriksen *et al.*, 2009). A study by Del Fattore *et al*. (2006) observed a statistically significant correlation between the high number of non-resorbing osteoclasts and the high number of osteoblasts observed in patients with autosomal recessive osteopetrosis. This was supported by the results of a study by Karsdal *et al*. (2008) which found that osteoclasts secrete signalling molecules that promote bone formation by osteoblasts. As the osteoclasts with which the number of osteoblasts was correlated were non-functioning, it has been suggested that it is the osteoclast itself and not its role in bone resorption which influences the action of osteoblasts

(Del Fattore *et al.*, 2006; Karsdal *et al.*, 2008). The secretion of signalling molecules by osteoclasts may therefore represent the "coupling factor" through which the rates of bone resorption and formation are linked (Karsdal *et al.*, 2008).

The stimulation of bone formation by osteoblasts is believed to be related to a number of anabolic signalling molecules. Although studies such as those by Karsdal *et al*. (2008) and Del Fattore *et al*. (2006) have found evidence that some of these products, such as TGF-β and Insulin-like Growth Factor- I (IGF-I) are secreted by osteoclasts, others may be released from the bone matrix during resorption (Henriksen *et al.*, 2009). Using murine models, compounds released from the bone through osteoclastic action have been shown to exert a positive effect on bone formation (Bikle *et al.*, 2002; Zhang *et al.*, 2002). Although IGF-I and TGF-β have been shown to influence the formation of new bone, the most widely studied influence on bone deposition is Parathyroid Hormone (PTH) (Rittmaster *et al.*, 2000; Martin and Sims, 2005).

Parathyroid hormone is primarily associated with bone resorption through its action to raise serum Ca^{2+} levels (Sampson, 1997; Martin and Sims, 2005). Studies have also shown that PTH is important for proper growth and maintenance of the juvenile and adult skeleton and has been used as a component of the treatment of osteoporosis in adults (Karaplis *et al.*, 1998; Rittmaster *et al.*, 2000; Sims and Gooi, 2008). The mechanisms by which PTH promotes bone growth are not clear, however it has been suggested that the action of PTH is mediated by other factors, including IGF-I (Bikle *et al.*, 2002). The actions of PTH in relation to bone formation are believed to be two-fold. Through the encouragement of osteoblastic precursor differentiation, it is suggested that PTH initially stimulates an increase in the number of differentiated osteoblasts and subsequently, through decreasing levels of osteoblast apoptosis, helps to maintain the number of osteoblasts available to secrete osteoid (Bikle *et al.*, 2002; Martin and Sims, 2005; Sims and Gooi, 2008).

The successful completion of a bone remodelling cycle requires the coordinated actions of multiple cell types and molecules including growth factors, hormones and enzymes (Hill, 1998). As obliteration of the epiphyseal scar is attributed to

bone turnover and remodelling, any factors that alter this process could influence the persistence of the epiphyseal scar in adult individuals (Garden, 1961). Consequently, it is necessary to consider the potential role of such factors in the persistence or obliteration of epiphyseal scars in adult individuals. Bone remodelling may be stimulated by physical (functional) or metabolic drivers. The following sections discuss some of the factors which may exert a significant effect on bone remodelling at a population level, in respect of their physical or metabolic influence on bone remodelling.

1.4.2 Physical influences on bone remodelling

1.4.2.1 Skeletal biomechanics, bone functional adaptation and strain adaptive response

The body of work relating to bone biomechanics has arisen from the analysis of bone in three contexts of increasing complexity: as a structure, a material and a biological system (Roesler, 1987). The origin of the study of bone biomechanics may be traced to the relationship between the anatomist Georg von Meyer and the engineer Carl Cullman, through which similarities between the cancellous structure of the proximal femur and the structure of a crane were noted (von Meyer, 2011). Based on this observation, it was postulated that the arrangement of trabeculae within this anatomical region occurred in response to the principal loading trajectories (Lee and Taylor, 1999). This theoretical approach was developed further by Julius Wolff, who applied mathematical formulae to describe the arrangement and adaptation of cancellous structure of the proximal femur under mechanical loading (Wolff, 1870; Lee and Taylor, 1999). This became known as "*Wolff's Law*" (Mullender and Huiskes, 1995).

The underlying suggestion that alteration to bone structure occurs in response to changes in mechanical loading has been supported by subsequent studies (Frost, 1987; Lanyon, 1987; Turner, 1991); however the mathematical basis of Wolff's law of bone transformation has been shown to be flawed (Lee and Taylor, 1999). In a reflection of the contemporary research climate, the theory suggested by Wolff was adapted by Roux who, in 1881, suggested the process of functional adaptation (Ruff *et al.*, 2006). This amended theory was based on two primary considerations: firstly that organisms were capable of adapting to changes in their

environment; and secondly that localised mechanical stresses may stimulate alteration to bone morphology (Ruff *et al.*, 2006).

The process of bone remodelling is a continuous balancing act between strength and mass where the goal is to achieve the strongest structure with the lowest mass, summarised as the "maximum-minimum principle" and is achieved by strain adaptive remodelling (Sissons and Kember, 1977; Roesler, 1987; Bokariya *et al.*, 2011). As different areas of the skeleton are exposed to varying levels of strain during normal function, the response of bone to extrinsic loading is thought to be site specific (Crowder and Austin, 2005; Modlesky *et al.*, 2011; Turunen *et al.*, 2013). This process is based on the premise of a biological negative feedback loop where the application of a force which exceeds the structural or mechanical competency of a bone stimulates modification of the osseous architecture to compensate for the increase in applied load (Frost, 1987; Turner, 1991; Frost, 1996; 1998a; 1998b; Frost *et al.*, 1998; Frost, 2003; Crowder and Austin, 2005). In contrast, exposure of the skeleton to mechanical forces which render the existing bone excessive, induce alteration of bone structure to reduce the mass to correspond with the forces imposed upon it such as occurs during immobilization due to injury or illness (Henderson *et al.*, 2002); or during space flight (Mack and Vogt, 1971).

In any feedback system, a mechanism must exist whereby changes in conditions are recognised and a subsequent response generated (Powell, 1961; Nowlan *et al.*, 2007). The manner in which strain changes produce a cellular response however was poorly understood until the late 1980s (Hollister *et al.*, 1991). Several authors have posited that osteocytes play a functional role in monitoring strain and through interactions with oestrogen receptors, stimulate bone alteration in a process termed cellular mechanotransduction (Hughes, 2010; Ubelaker and Zarenko, 2012).

Osteocytes account for approximately 90-95% of bone cells and are connected to each other through a network of dendritic processes, located within canaliculi (Acsadi and Nemeskeri, 1970; Cowin, 2002). As osteocytes are also connected to osteoblasts and bone lining cells through the cell processes, they are ideally placed

to affect a change in the bone remodelling in response to external stimuli (Cowin, 2002).

Although the exact mechanism behind cellular mechanotransduction is poorly understood, two theories dominate the literature on the subject (Turner and Pavalko, 1998). Firstly, bone fluid flow theory suggests that when an external load is applied to bone, the strain causes micro-deformations to occur in the calcified bone matrix (Mullender and Huiskes, 1995; Mullender *et al.*, 2004; Dedouit *et al.*, 2008). This increases the pressure of the interstitial fluid between osteocytes, which amplifies the mechanical signal sensed by the osteocytes and thereby stimulates the secretion of signalling molecules by the osteocytes and thereby influences the rate of bone remodelling (Cowin, 2002; Dedouit *et al.*, 2008).

The second hypothesis regarding the mechanism of cellular mechanotransduction suggests that mechanical stimulation of osteocytes causes the release of a signalling molecule which binds to oestrogen receptors resulting in the release of oestrogen, which is known to exert a positive effect on bone formation (McCormick and Stewart, 1988). The continued reduction in bone mineral associated with age suggests that mechanical stimulation of bone may promote remodelling through an intermediate factor which exhibits an age related decline, such as oestrogen (McCormick and Stewart, 1988).

Irrespective of the mechanism by which it occurs, it is clear that dynamic mechanical stimulation of the skeleton produces an osteogenic response which improves bone mineral density (BMD) and facilitates the adaptation of the skeleton to confirm to its structural requirements (Frost, 1987; Ehrlich and Lanyon, 2002; Egan *et al.*, 2006). Similarly, immobility can result in disuse injuries which stem from a reduction in bone mass as a response to the lack of mechanical stimulation (Mack and Vogt, 1971; Vose, 1974).

1.4.3 Metabolic influences on bone remodelling

1.4.3.1 Factors related to biological sex

The interplay between body mass and bone mineral density has been discussed in various contexts, including that of biological sex and the influence of endocrine factors. In general, due to the influence of androgens, and testosterone in

particular, male individuals tend to have a greater total body mass and lean body mass than females (Düppe *et al.*, 1997). This equates to a larger physical strain imposed on the skeleton and therefore a greater mechanical load resulting in increased bone remodelling (Hsieh *et al.*, 2001). The increase that is observed in males in comparison to females could be counteracted by the higher levels of oestrogens in females, a hormone which is known to increase bone mineral density (Zaman *et al.*, 2000). Females tend to exhibit a higher percentage of body fat than males. This facilitates the conversion of adrenal androgens to oestrogens, further positively influencing bone remodelling (Taaffe *et al.*, 2001; Frank, 2003).

Sex hormones are important regulators of bone metabolism and as a result, the roles of both oestrogens and androgens will be discussed separately.

Oestrogens

The effect of sex steroids on bone was first presented by Fuller Albright in 1948, who, through a study concerning the incidence of oophorectomy within a group of osteoporosis patients, discovered a prevalence rate of the condition that was higher than would be expected in the general population (Compston, 2001; Balasch, 2003; Järvinen *et al.*, 2003). From this, it was proposed that oestrogen stimulated osteoblast function and consequently, its absence resulted in a net decrease of bone formation and a concomitant decrease in BMD, as was observed in oophorectomised study subjects (Compston, 2001; Järvinen *et al.*, 2003).

The mechanism of action of oestrogen on bone remodelling is believed to directly influence osteoblasts, through interaction with receptors on the cell surface and indirectly via stimulation of osteoclast mediator molecules (Kameda *et al.*, 1997; Krassas and Papadopoulou, 2001; Nilsson *et al.*, 2001). These have been suggested to include stimulation of the production of mediator molecules, such as OPG, and a reduction in the secretion of Colony Stimulating Factor-1 (CSF-1) by osteoblasts, thereby increasing osteoblastic differentiation from progenitor cells (Compston, 2001; Eastell, 2005).

It has also been suggested that in addition to their positive influence on osteoblast function, oestrogens also exert a suppressive effect on osteoclasts through the mediation of cell apoptosis, resulting in an alteration to the rate of bone resorption

(Balasch, 2003; Eastell, 2005). Through these interactions, oestrogen influences both osteoclastogenesis and cellular apoptosis (Bland, 2000).

Androgens

For many years, androgens were regarded as the male sex hormones, just as oestrogens were considered to be the female sex hormones (Frank, 2003). Consequently, these hormones were believed to be solely responsible for bone growth and development within their relative sex (Frank, 1995). Both hormone types however are found in males and females and both appear to have an effect on bone metabolism and turnover (Notelovitz, 2002; Frank, 2003).

Androgens primarily encourage the proliferation of osteoblasts, they also encourage the apoptosis of osteoclasts and inhibit the apoptosis of osteoblasts (Notelovitz, 2002; Balasch, 2003). The effect of androgens on bone has been investigated largely in relation to the skeletal manifestations of clinical conditions which result in abnormal levels of systemic androgens, including Polycystic Ovary Syndrome (PCOS) (Zborowski *et al.*, 2000) and secreting ovarian tumours (Balasch, 2003; Castelo-Branco *et al.*, 2003), where effects are known to include a higher than normal BMD. In males, the effects of androgens on the skeleton have been investigated in patients with androgen insensitivity syndrome (AIS), the effects of which are known to include irregular bone metabolism, increased remodelling rate and delayed epiphyseal fusion (Hofbauer and Khosla, 1999; Marcus *et al.*, 2000).

Through investigations regarding AIS, it is apparent that androgen receptors are located on several types of cell including hypertrophic chondrocytes and osteocytes (Hofbauer and Khosla, 1999; Danilovic *et al.*, 2007). The osseous manifestations of AIS suggest that the roles of androgens within the skeletal system include initiation of epiphyseal fusion. This has been supported by the findings of a study of Turner's Syndrome patients where epiphyseal fusion did not commence until androgen therapy had been administered (Even *et al.*, 1998). The studies of AIS patients also suggest that a reduction in androgen sensitivity results in a decrease in BMD, potentially as a result of a reduction in peak bone mass (Hofbauer and Khosla, 1999). Conversely, the higher level of BMD found in

individuals with excess levels of systemic androgens reflects the positive effect of androgens on osteoblasts (Compston, 2001).

1.4.3.2 Factors related to chronological age

The effect of age on bone remodelling and as a result, BMD, can be considered as the cumulative effect of multiple factors to which the individual has been exposed during their life time. Consequently, it is difficult to consider the parameter of "age" as a single entity. Despite this, it is possible to characterise the rates of bone remodelling commonly observed during specific phases of life.

The literature relating to the effects of age on bone remodelling rate in healthy individuals is sparse. Several studies have been conducted to assess the levels of biochemical markers of bone formation (including osteocalcin, bone alkaline phosphatase and procollagen Type 1 N-terminal and C-terminal propeptides); and bone resorption (including hydroxyproline, N-Telopeptide and C-Telopeptide) (Schoenau and Rauch, 2009; Jürimäe, 2010; Walsh *et al.*, 2010; Huang *et al.*, 2011; Faje *et al.*, 2012). Although these markers are indicative of bone turnover, it is not possible to distinguish between markers of skeletal growth and those of remodelling in adolescent individuals (Jürimäe, 2010; Huang *et al.*, 2011). This may partially explain the high levels of "markers of bone remodelling" observed during adolescence (Walsh *et al.*, 2010; Huang *et al.*, 2011). During this time, the rate at which bone remodels is related to numerous factors including levels of sex steroids and levels of vitamin D (Huang *et al.*, 2011).

The levels of biochemical "markers of bone remodelling" remain relatively high during the period of net bone mass acquisition which continues into the third decade (Walsh *et al.*, 2010). Relatively few studies have considered the remodelling of bone in healthy adults, tending to favour investigations related to osteoporosis. Within adult individuals who have attained maximum bone mass, maintenance of calcium homeostasis and of structural competency of the skeleton become the primary drivers of bone remodelling. The influence of both endogenous and exogenous factors may cause deviation from the norm resulting in an alteration to the rate of remodelling. These factors could include hormonal status (including pregnancy and lactation in females) (Wardlaw and Pike, 1986);

nutritional or health status (Prentice, 1997; Nelson, 2000); physical activity and mechanical loading (Mack and Vogt, 1971; Frost, 1987; Branca and Vatueña, 2001; Murphy and Carroll, 2003; Bass, 2012); consumption of alcohol (Schnitzler and Solomon, 1984; Rico, 1990; Moniz, 1994; Sampson, 1997; Ganry *et al.*, 2000; Rapuri *et al.*, 2000a; Turner, 2000; Turner *et al.*, 2001; Callaci *et al.*, 2004; Chakkalakal, 2005; Maurel *et al.*, 2012; Turner *et al.*, 2012); caffeine (Barger-Lux *et al.*, 1990; Heaney, 2002; Ilich *et al.*, 2002); cigarette smoking (Raikin *et al.*, 1998; Fung *et al.*, 1999; Hollinger *et al.*, 1999; Iwaniec *et al.*, 2001; Gullihorn *et al.*, 2005; Rothem *et al.*, 2009; Hapidin *et al.*, 2011; Kim *et al.*, 2012) and the effects of some medications (Dumont *et al.*, 2000; Head *et al.*, 2001). It has been suggested that socioeconomic status may also influence bone turnover, however this is likely to be related to resource acquisition, such as access to health care and adequate nutritional intake (Garn *et al.*, 1973b; Demeter *et al.*, 2007; Crandall *et al.*, 2012).

1.4.3.3 Toxins

As part of everyday life, people expose themselves to toxins, which, although perhaps considered normal or mainstream, may alter the normal metabolic processes that occur within the human body. As the number of substances to which this could refer is vast, this discussion has been restricted to the three most widely available substances that have been investigated in relation to their effect on bone remodelling.

Alcohol

In 2007 alone, the cost of prescription medications for the treatment of alcohol dependency in England totalled £2.38 million (The NHS Information Centre and Lifestyles Statistics, 2010). Considered a major social issue, the over-consumption of alcohol and its associated effects on health form the base for many research projects (Moniz, 1994; Kimble, 1997; Sampson, 1997; Rapuri *et al.*, 2000a; Turner, 2000; Turner *et al.*, 2001; Ilich *et al.*, 2002; Hefferan *et al.*, 2003; Callaci *et al.*, 2004; Chakkalakal, 2005; Maurel *et al.*, 2012).

Excessive consumption of alcohol is known to result in a reduction of bone mass and the development of osteopenia or osteoporosis (Turner *et al.*, 2001; Chakkalakal, 2005; Maddalozzo *et al.*, 2009). The mechanism by which alcohol

related bone loss occurs is believed to be related to the suppression of new bone formation by osteoblasts through either direct or indirect pathways (Turner, 2000; Callaci *et al.*, 2004; Maurel *et al.*, 2012). A histological study by Schnitzler and Solomon (1984) observed that the mean trabecular volume and thickness were lower in individuals with a history of heavy alcohol consumption compared with those who consumed moderate levels of alcohol. The results of this study also suggested that long term alcohol use results in an increase in the rate of bone resorption and a concomitant decrease in the rate of bone formation, thus, resulting in a net loss of bone (Schnitzler and Solomon, 1984). The findings of this study are supported by the results of a biochemical analysis of markers of bone remodelling conducted by Labib *et al*. (1989) who found that the levels of serum osteocalcin, a vitamin K dependent marker of bone formation, were lower in individuals who had a history of high alcohol intake in relation to age-matched controls. Reports within the literature suggest that the reduction in osteoblast activity may be due to a reduction in the production of Leptin, a protein which stimulates the differentiation of osteoblasts from their progenitor cells (Maurel *et al.*, 2012; Turner *et al.*, 2012).

Although some reports have been made relating to the potential increase in osteoclastic effects as a result of high levels of alcohol consumption, the relationship between these factors is not as constant as that observed in the interaction between alcohol and osteoblasts (Turner, 2000; Callaci *et al.*, 2004). It appears to be the consensus that the progressive loss of bone mineral related to alcohol consumption is as a result of repeated instances of small net loss in bone during each round of the remodelling cycle. In addition to the possible direct effects of alcohol on osteoblasts, there are numerous suggestions in the literature that many of the influences associated with alcohol-related osteopenia are as a result of metabolic responses to other lifestyle factors that often accompany heavy alcohol consumption, for instance low levels of physical activity and malnutrition (Rico, 1990; Turner, 2000; Chakkalakal, 2005; Maurel *et al.*, 2012).

The detrimental effects of high levels of alcohol consumption on bone have been reported and accepted for a number of years, however with the advent of the binge drinking culture, it is necessary to consider whether a dose dependent response is

involved in alcohol-induced bone loss (Callaci *et al.*, 2004; Maurel *et al.*, 2012). While heavy consumption of alcohol is reported to result in bone loss, small quantities may confer a beneficial or protective effect on bone mineral density as a result of a reduced bone remodelling rate (Ilich *et al.*, 2002; Maurel *et al.*, 2012). The effect of moderate levels of alcohol on bone mineral density is reported to be dependent on factors including the sex of the individual and their hormonal status. For example, the effect of moderate alcohol intake (up to three glasses of wine per day) in postmenopausal females has been shown to confer a positive influence on BMD (Ganry *et al.*, 2000). As highlighted in the potential relationship between bone loss and high alcohol intake and associated factors, it is noted by Ganry *et al*. (2000) that the individuals included in their study who reported consuming moderate quantities of alcohol were more likely to be of higher socioeconomic and health statuses. Consequently, the beneficial effects on BMD that were correlated with moderate levels of alcohol consumption could also be related to other environmental factors. The possible positive effects of small to moderate quantities of alcohol on BMD and bone remodelling therefore remain a matter of debate.

Cigarette smoking

Cigarette smoke is a complex mixture of approximately 4000 chemicals including nicotine, carbon monoxide, carbon dioxide, nitrogen, ammonia and hydrogen cyanide (Gullihorn *et al.*, 2005; Sloan *et al.*, 2010; Kim *et al.*, 2012). Consequently, it is difficult to distinguish the effects of the individual components on the rate of bone remodelling. Cigarette smoking is associated with an increased risk of developing osteoporosis and associated fractures of the forearm, proximal femur and vertebrae (Hopper and Seeman, 1994; Rapuri *et al.*, 2000b; Iwaniec *et al.*, 2001); however the mechanism by which bone loss occurs in relation to cigarette smoking is not fully understood (Hopper and Seeman, 1994; Krall and Dawson-Hughes, 1999; Rapuri *et al.*, 2000b; Ward and Klesges, 2001). A number of potential direct and indirect mechanisms have been postulated in the literature including the suppression of osteoblastic activity, increased oestrogen metabolism, decrease in intestinal calcium absorption and alterations to hormonal secretion (Pocock *et al.*, 1989; Hollenbach *et al.*, 1993; Vogel *et al.*, 1997; Krall and Dawson-

Hughes, 1999; Gullihorn *et al.*, 2005). It is also noted in the literature that the observed influences of cigarette smoking on bone may be related to confounding variables including body mass, nutrition and alcohol consumption (Pocock *et al.*, 1989). The results obtained by Vogel *et al.* (1997) indicated that the effect of smoking related bone loss may be more pronounced in cancellous bone than in cortical bone. This may be explained by the greater surface area over which remodelling can occur in a cancellous structure compared with cortical bone.

As the primary addictive, physiologically active agent within cigarette tobacco, several studies have considered the effect of nicotine on bone in both animal and human models (Raikin *et al.*, 1998; Fung *et al.*, 1999; Hollinger *et al.*, 1999; Iwaniec *et al.*, 2001; Gullihorn *et al.*, 2005; Rothem *et al.*, 2009; Hapidin *et al.*, 2011; Kim *et al.*, 2012). Despite the extensive research that has considered the role of nicotine in bone loss related to cigarette smoking, the matter remains a source of debate within the literature (Iwaniec *et al.*, 2001). It has been proposed that the effects of nicotine on BMD may arise through the interaction of nicotine and its receptor, which is located on the surface of osteoblasts and through the indirect effects associated with insufficient vascularisation of bone (Sloan *et al.*, 2010). A study by Rothem *et al.* (2009) suggested that in the presence of high levels of nicotine, binding of the chemical to its receptor on the surface of osteoblasts results in a decrease in the levels of Type 1 collagen and osteocalcin secreted. This then causes a reduction in osteoblastic differentiation and a concomitant decrease in bone formation.

As heavy smokers have been observed to exhibit increased levels of serum osteocalcin and N-Telopeptide/creatinine ratio, which are markers of bone formation and resorption respectively, disruption to the remodelling cycle due to the effects of nicotine has also been suggested as a factor in smoking related bone loss (Rapuri *et al.*, 2000b). This suggests that a higher rate of bone remodelling occurred in heavy smokers than was observed in either light or non-smokers and may be attributed to an inhibitory effect of nicotine on bone cell maturation and function (Rothem *et al.*, 2009). This may result in a statistically significant loss of bone mineral compared with non-smokers, particularly in postmenopausal females and elderly males (Hollenbach *et al.*, 1993; Vogel *et al.*, 1997; Rapuri *et al.*,

2000b; Iwaniec *et al.*, 2001; Ward and Klesges, 2001). These results however are in conflict with those of several other authors who found that in small doses, nicotine exerted a stimulatory effect on bone cell proliferation and metabolism (Gullihorn *et al.*, 2005; Rothem *et al.*, 2009; Kim *et al.*, 2012).

Caffeine

Caffeine, as a naturally occurring stimulant, has been consumed in various forms across the globe (Heaney, 2002). Work conducted during the 1980s and 1990s showed that ingestion of dietary caffeine resulted in an increased level of urinary calcium excretion, suggesting an interaction with bone (Massey and Wise, 1984; Bergman *et al.*, 1990). There are multiple pathways through which caffeine could influence the strength of bone, including a direct interaction with bone remodelling or decrease in bone mass (Heaney, 2002). The potential relationship between caffeine and bone is believed to relate to its interactions with other metabolites, specifically calcium (Barger-Lux *et al.*, 1990). As the skeleton represents the vast majority of the calcium stored within the body, any alteration to serum levels of Ca^{2+} will induce a change in the rate of remodelling to restore the normal calcium levels (Hernandez-Avila *et al.*, 1991; Demirbag *et al.*, 2006). This theory is not supported by the results of a study by Barger-Lux *et al*. (1990) which found no statistically significant variation in the level of calcium found in the urine of participants taking 400mg of caffeine per day compared with those on a placebo. This was supported by the results of Sakamoto *et al*. (2001) who found, in murine models, that high caffeine consumption was not related to an increase in calcium excretion, however a statistically significant increase in urinary phosphorus was found after 140 days in the animals to whom coffee was fed.

1.4.3.4 Medications and hormone supplementation

The number of prescriptions dispensed in Scotland has undergone an annual increase of approximately 3% since 2010, reaching 96.6 million in 2012 (NHS Scotland, 2013). This number does not include the sale of over-the-counter medications, which according to the Proprietary Association of Great Britain generated £2333 million in 2011 (2012). Due to the prevalence of medication use within the general population, it is necessary to consider the potential influences

that some more commonly used pharmaceuticals may have on bone remodelling and therefore their prospective effect on the persistence of the epiphyseal scar.

Analgesic medications

The range of medications used to control pain varies depending on its severity, cause and duration; and are categorised according to their strength (Vestergaard, 2008). As some of the most commonly used analgesics, it is not surprising that the possible skeletal side effects of *acetaminophen*, otherwise known as *Paracetamol* and members of the *non-steroidal anti-inflammatory drugs* (NSAIDs) group have received attention in the literature (Dumont *et al.*, 2000; Head *et al.*, 2001; Beck *et al.*, 2003; Bergenstock *et al.*, 2005; Pountos *et al.*, 2008; Cottrell *et al.*, 2009; García-Martínez *et al.*, 2011; Williams *et al.*, 2011).

The analgesic and antipyretic effects of *acetaminophen* are directed through the endocannabinoid and cyclooxygenase (COX) pathways, through which, modification of osteoclastic action via Type 2 cannabinoid receptors may occur, as observed during hypothalamic regulation of bone remodelling (Driessler and Baldock, 2010; Williams *et al.*, 2011). It is also suggested that acetaminophen decreases the level of prostaglandins (Head *et al.*, 2001). These lipids, which form in response to the cyclooxygenase-2 enzyme (COX-2) act as local mediators in load-induced bone remodelling (Raisz, 1999; Blackwell *et al.*, 2010; Williams *et al.*, 2011). Non-steroidal anti-inflammatory drugs have also been shown to inhibit the production of prostaglandins, particularly in relation to wound and fracture healing where continued drug administration has been found to result in delayed recovery (Dumont *et al.*, 2000; Beck *et al.*, 2003; Giordano *et al.*, 2003). Prostaglandins are known to promote bone formation (Blackwell *et al.*, 2010). Consequently, an alteration to the production of these compounds may result in a decrease in bone formation (Williams *et al.*, 2011).

Although not as commonly used as the medications discussed in the previous paragraphs, long term treatment or use of opiates may influence the rate of bone remodelling or the BMD of the individual (Dürsteler-Macfarland *et al.*, 2011). According to the review of literature conducted by Vestergaard (2008), no studies have been conducted which specifically examine the influence of opioids on bone

cells, however opioid receptors have been noted on osteoblasts, suggesting a potential role of endogenous opioids in the mediation of bone remodelling (Rosen *et al.*, 1998). Several studies have examined the potential link between exogenous opiates, low BMD and impaired bone repair (Motamedi *et al.*, 2005; Kim *et al.*, 2006; Dürsteler-Macfarland *et al.*, 2011; Gozashti *et al.*, 2011). Although a direct relationship between exogenous opiates and BMD has not been found, it is suggested that administration of these drugs results in abnormal production of sex steroids and other hormones of the hypothalamic-pituitary-gonadal axis (Motamedi *et al.*, 2005; Dürsteler-Macfarland *et al.*, 2011; Gozashti *et al.*, 2011). As these hormones exert an effect on bone remodelling (see section 1.4.3.1), it is reasonable to suggest that long term use of opiates, whether in a therapeutic or non-therapeutic environment, will affect bone remodelling and BMD (Kim *et al.*, 2006).

In the context of non-therapeutic administration of opiates, it is necessary to consider that long term drug users are more likely to express high risk behaviours, some of which, for example smoking or alcohol abuse, may be related to a reduction in BMD and a concomitant increase in bone remodelling rate (see section 1.4.3.3) (Turner *et al.*, 2001; Gozashti *et al.*, 2011). Individuals who are addicted to opiates are also more likely to have a poor diet and low levels of dietary calcium and other vitamins and minerals necessary for normal bone remodelling (Kim *et al.*, 2006; Gozashti *et al.*, 2011). As a result, there may be numerous factors that could influence the BMD and bone remodelling rate of a habitual opiate user other than those either directly or indirectly related to the drug itself (Dürsteler-Macfarland *et al.*, 2011).

Hormonal contraceptives and hormone replacement therapy

Studies have been carried out to examine the effect of hormonal contraceptives on bone health in both adolescent and premenopausal adult females (Goldsmith, 1975; Pasco *et al.*, 2000; Berenson *et al.*, 2001; Ott *et al.*, 2001; Perrotti *et al.*, 2001; Wanichsetakul *et al.*, 2002; Elgán *et al.*, 2003; Cromer *et al.*, 2004; Lara-Torre *et al.*, 2004; Liu and Lebrun, 2006; Hartard *et al.*, 2007). It has been suggested that administration of oral contraceptives, including both the combination pill and "mini-pill" may result in a small increase in BMD or have no overall effect on bone

health (see section 1.4.3.1) (Pasco *et al.*, 2000; Perrotti *et al.*, 2001; Golden *et al.*, 2002; Wanichsetakul *et al.*, 2002; Elgán *et al.*, 2003; Burkman *et al.*, 2004; Frye, 2006).

In contrast to oral contraceptives, the use of depot medroxyprogesterone acetate (DMPA) has been associated with a loss in BMD , although this research is largely restricted to those skeletal regions examined during the clinical monitoring of osteoporosis, such as the lumbar spine and the proximal femur(Wanichsetakul *et al.*, 2002; Cromer *et al.*, 2004). The detrimental influence of DMPA on bone health may be mitigated by restricting the duration of periods of administration. This has been reinforced in the United States of America, where the Federal Drug Administration (FDA) recommend that DMPA is not used for periods exceeding 2 years, although the justification for this limitation is not universally accepted (Cromer *et al.*, 2006; Kaunitz *et al.*, 2008). The adverse effects of DMPA on bone have been associated with its anti-oestrogenic effects which occur through suppression of oestrogen receptors and of androgenic secretion, thereby increasing the rate of bone turnover (Di Carlo *et al.*, 1984; Dowsett *et al.*, 1987).

It is apparent that alterations to the level of circulating oestrogen may significantly affect the rate of bone remodelling and BMD. Although administration of oral contraceptives may or may not positively influence BMD in premenopausal women, the beneficial effects of long term hormone replacement therapy (HRT) have been largely accepted (Castelo-Branco *et al.*, 1992; Hannon *et al.*, 1998; Gambacciani *et al.*, 2003; Popp *et al.*, 2006). Age associated bone loss occurs as a result of decreasing levels of oestrogen production and a subsequent increase in the rate of bone turnover (Castelo-Branco *et al.*, 1992). This may be reduced through the prescription of HRT, which increases the level of serum oestrogen levels (Compston, 1992). As oestrogen is required for the regulation of the bone remodelling cycle and the maintenance of bone mass, administration of HRT medications results in a reduction in the rate of bone turnover and a concomitant moderation of bone loss (Delmas, 1997).

Glucocorticoids

Glucocorticoids are a group of steroid hormones frequently prescribed as part of the clinical management of auto-immune conditions (Rauch *et al.*, 2010), allergies and asthma (Avioli, 1993; Rehman and Lane, 2003). According to Asthma UK, (2011) 5.4 million people in the United Kingdom were receiving treatment for asthma in 2011, including 1.1 million children. Due to the possible implications for child public health, attention has been paid to the adverse effects of glucocorticoid treatment for asthma on bone growth and development (Avioli, 1993).

The results of a study conducted by Wolthers and Pedersen (1990) suggested that ingestion of the glucocorticoid *Prednisolone* was associated with a decrease in growth velocity in all children to whom the active substance was given compared with the placebo group. The mechanism of action of glucocorticoids on bone is related to their influence on the apoptosis of osteoblasts and the length of the cell life of osteoclasts, resulting in a bias towards resorption in the remodelling cycle, thereby resulting in a decrease in BMD (O' Brien *et al.*, 2003).

An alternative pathway for the effect of glucocorticoids on bone could be linked to their interaction with chondrocytes in relation to the production of vascular endothelial growth factor (VEGF) (Koedam *et al.*, 2002; Maes *et al.*, 2002). The study by Koedam *et al.* (2002) suggested that administration of glucocorticoids results in a reduction in the production of VEGF, which is required for angiogenesis. As vascular invasion is required for proper mineralisation of osteoid, compromise to the signals which stimulate this process could therefore interfere with the normal bone remodelling process (Gerber and Ferrara, 2000; Kanczler and Oreffo, 2008).

1.4.3.5 Parathyroid Hormone

The effects of PTH on bone has been widely documented and has been noted to induce dose dependent responses in bone metabolism whereby intermittent administration results in an increase in BMD while continuous prolonged exposure exerts a deleterious effect on bone (Schneider *et al.*, 2012). The adverse effects of long term exposure to PTH have been observed in patients suffering from hyperparathyroidism, a condition which is associated with higher than normal

levels of bone resorption (Kollars *et al.*, 2005; Bedi *et al.*, 2010). In contrast to this, the associated effects of hypoparathyroidism or pseudohypoparathyroidism where either low levels of PTH or PTH insensitivity are encountered, an individual may be found to exhibit an abnormally elevated BMD and a concomitantly low bone remodelling rate, characterised by hypocalcaemia (Ma and Cockram, 2009; Bedi *et al.*, 2010; Amrein *et al.*, 2011)

Although the biphasic response of bone to PTH is generally accepted, the mechanism for the anabolic effects of intermittent PTH exposure is not fully understood (Robling *et al.*, 2011). Parathyroid hormone is known to stimulate osteoblast proliferation and differentiation from their precursor cells and reduce osteoblast apoptosis, thereby facilitating an increase in bone formation (Robling *et al.*, 2011). This occurs through the interaction between PTH and its PTHrP receptor, which is expressed by cells of the osteoblastic lineage (Bedi *et al.*, 2010). In addition to the effects of PTH on osteoblasts, it has been reported that PTH increases the rate of osteoclastic action, resulting in an increase in the overall bone remodelling rate (Robling *et al.*, 2011). It is reported that the effect of PTH on bone resorption results from the stimulation of the secretion of RANKL and the inhibition of the secretion of OPG by osteoblasts (Bedi *et al.*, 2010). It has also been suggested that through its interaction with VEGF, PTH influences the distribution of vascular structures within areas of new bone formation, thus influencing the provision of nutrients, thereby creating an environment conducive to bone formation (Prisby *et al.*, 2011).

1.5 Summary

According to Article 1 of the United Nations Convention on the Rights of the Child, a juvenile is defined as any individual under the age of 18 years (United Nations, 1989). Although this definition is absolute, skeletal growth and development, maturation and maintenance may be regarded as contiguous phases, the distinctions between which are somewhat less clear than the legal definition suggests (Scheuer, 2002). As a result of the genetic, biological and environmental influences to which an individual is exposed, the timing and duration of these phases may vary between individuals and populations (Rikhasor *et al.*, 1999; Schmeling *et al.*, 2005a). These factors not only affect the progress of skeletal

maturation, but may also influence the rate at which bone remodelling occurs. It is therefore necessary that any estimation of skeletal age is based on an approach which has been found to be accurate and reliable, particularly if it is to be undertaken for medico-legal purposes (The Law Commission, 2011). Examination of the literature has shown that while many approaches to skeletal age estimation have undergone testing, those based on the foot and ankle have, until recently, been neglected (Andersen, 1971; Cole *et al.*, 1988; Büken *et al.*, 2009; Modi *et al.*, 2009; Hackman and Black, 2013a; 2013b; Hackman *et al.*, 2013). The question of whether the two existing approaches to skeletal age estimation from this region are fit for purpose must be asked. The initial phase of this study will add to the existing literature relating to this question.

It is apparent that bone remodelling may be susceptible to alteration by numerous factors that are diverse, both in their timing and mechanism of action. As a result of the constant process of bone remodelling, it has been suggested that the epiphyseal scar may become obliterated after a period of time following epiphyseal fusion (Garden, 1961; Workshop of European Anthropologists, 1980). Despite several contradictory reports, the presence of epiphyseal scars continues to be interpreted as an indication that epiphyseal fusion may have recently occurred (MacLaughlin, 1987; Whitaker *et al.*, 2002; Schmidt *et al.*, 2008; Baumann *et al.*, 2009; Kellinghaus *et al.*, 2010; Weiss *et al.*, 2012). Although this may be true of some skeletal regions, the site specific nature of bone remodelling may suggest that this may not be the case in all areas. The second phase of this study will address lingering questions relating to the persistence or obliteration of epiphyseal scars in five anatomical regions and augment the existing literature relating to the persistence or obliteration of this feature in adult individuals.

2 Materials and Methods

Due to the legal and ethical constraints surrounding the use of x-rays and the potential dangers of their repeated application, it would not have been feasible to perform this study using a longitudinal sample (Brenner *et al.*, 2003). As a result, this study only utilised pre-existing radiographs that were taken during the course of clinical assessment or treatment. As the radiographs used in this study represent cross-sections of the juvenile and adult populations of Tayside, it is prudent to consider the demographics of the population from which they originate (Schmeling *et al.*, 2000).

2.1 Study sample demographics

Tayside NHS Trust supplies medical services within three local authorities: Perth and Kinross, Angus and Dundee City. The population size across these three local authority areas is approximately 386,600 (Directorate of Change and Innovation, 2004). The breakdown of this population by local authority is illustrated in Figure 2.1. The actual number of patients covered by Tayside NHS Trust may be marginally less than that of the total population due to a geographical overlap with Fife NHS Trust; however due to local agriculture and the high regional student population, there is a large temporary population, which may or may not be accounted for in these data (Directorate of Change and Innovation, 2004).

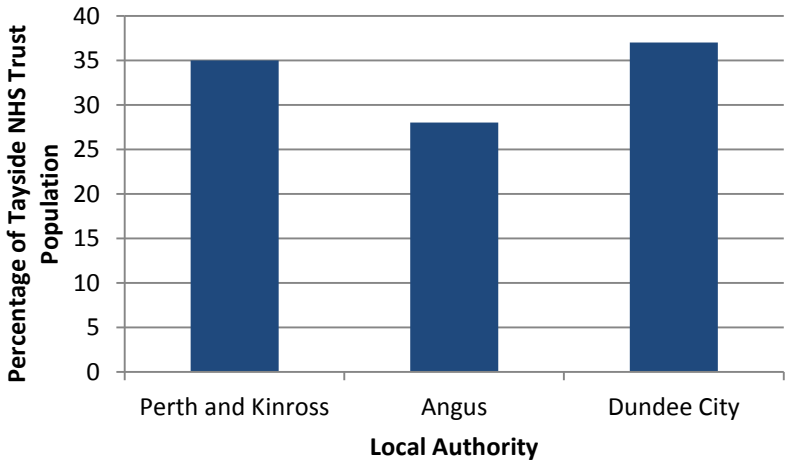

Figure 2.1: Distribution of the Tayside NHS population according to local authority

Within Dundee City local authority, 24% of families are in relative poverty as defined by the Scottish Government (2010); 27.6% of primary school aged

children and 21.2% of children at secondary school are entitled to free school meals (Directorate of Change and Innovation, 2004). This is slightly higher than in Angus local authority where 19% of families are in poverty; however the poverty level within both local authorities is above the national average of ~16% (The Scottish Government, 2010).

According to data from the 2001 census only 1.9% of the Tayside population is of non-European ancestry (Directorate of Change and Innovation, 2004). This may be influenced by the large number of students and migrant agricultural workers who temporarily reside in the region; however it is unlikely that this minor alteration to the demographic profile will affect the outcomes of this study.

2.2 Study phase 1 - materials and methods

2.2.1 Study sample

The radiographs were obtained from Tayside NHS Trust at Ninewells Hospital, Dundee. The data collected for use in this study included the biological sex and the date of birth (DOB) of each individual and the date on which each image was acquired. This has been termed the date of image acquisition (DOI). Chronological age was calculated by subtracting the DOB from the DOI. The chronological age of the individual was then converted from years to months.

As the images used were obtained for clinical analysis of injury, the appropriate ethical consents were obtained from NHS Tayside (see appendix B). All data was stored in accordance with the Data Protection Act (United Kingdom Government, 1998).

As images were obtained from a clinical database, all individuals who exhibited evidence of or were recorded as fitting the following criteria were omitted from the sample:

- A history of chronic illness or disease e.g. cancer
- A history of pathological conditions, including hip dysplasia and/or any medical conditions which may have affected the development of the long bones.

- Undergone precocious growth or medical problems during puberty, including delayed or precocious puberty.
- A past fracture.

Depending on whether an image was taken as digitised or film radiograph, it was either downloaded from the digital radiograph system or a photograph was taken of the image using an 8 megapixel digital camera and a light box. To enable a complete analysis of the foot and ankle, both anterior-posterior and lateral view radiographs were collected. Examples of the radiographic images used in these analyses are presented in Figure 2.2 .

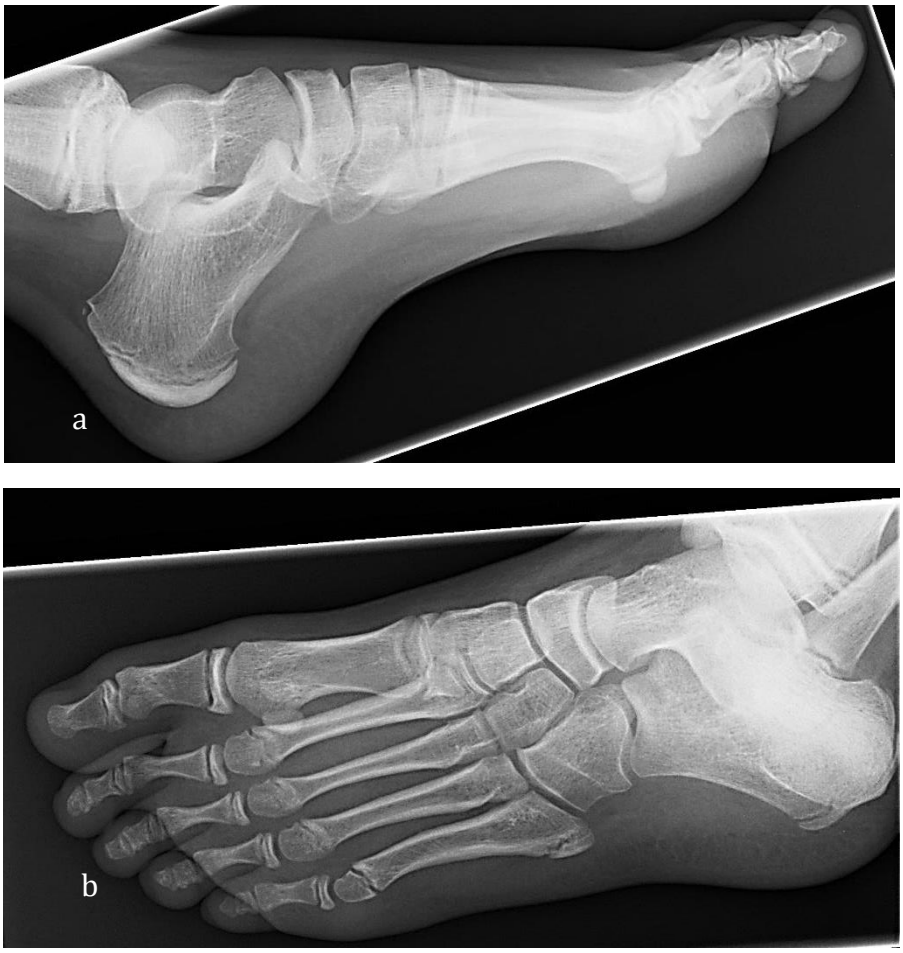

Figure 2.2: (a) Lateral and (b) anterior-posterior view radiographs of left foot from a female aged 12 years

Each individual was assigned a unique reference number (URN) based on the sex of the individual, the side from which the radiograph was taken, and a sequential number e.g. MRF1 (Male right foot: number 1).

A final sample of radiographs from 534 individuals comprising 224 females (170 lefts and 54 rights) and 310 males (196 lefts and 114 rights) aged between birth and 18 years was collected. Images from both sides of the body were represented in the sample. No individual was represented by more than one set of radiographs. The distribution of the sample used in the initial phase of this study is presented in Figure 2.3 according to biological sex, side of the body and chronological age.

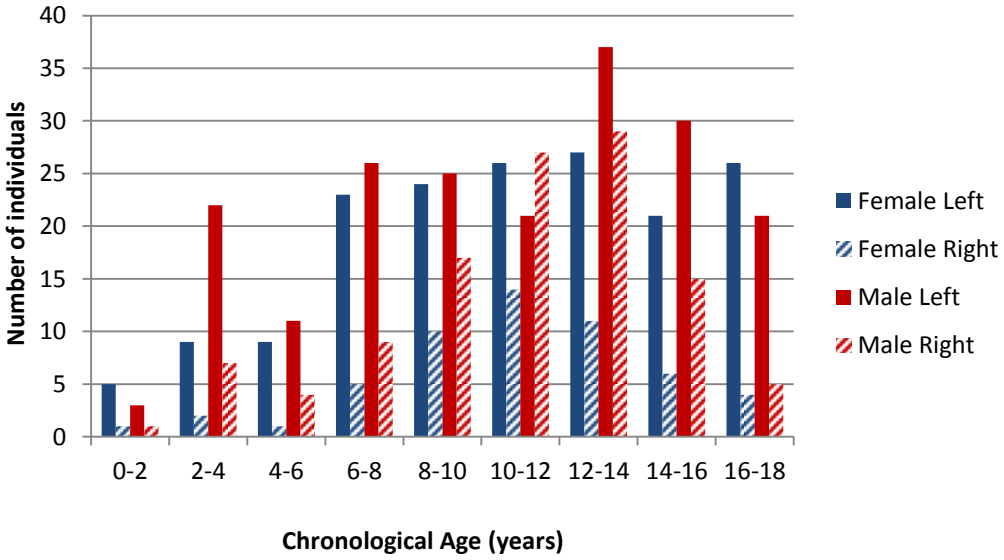

Figure 2.3: Distribution of the sample of foot radiographs according to chronological age, biological sex and side of the body

Although no literature has specifically examined bilateral asymmetry in the timing of appearance of centres of ossification in the foot, the absence of significant discrepancies in skeletal maturation between the left and right sides of the body has been shown in other skeletal areas including the hand and wrist, elbow and knee (Dreizen *et al.*, 1957; Loder *et al.*, 1993; Cheng *et al.*, 1998; O'Connor *et al.*, 2008; Hackman and Black, 2012). There is no reason therefore to presume that variation in skeletal maturity between the left and right feet would occur. Consequently, images from the left and right sides of the body will be grouped for analysis during the first phase of this study.

2.2.2 Approaches to skeletal age estimation from the foot and ankle

The two methods that were tested in the initial phase of this study were:

- Scoring System for Estimating Age in the Foot Skeleton (Whitaker *et al.*, 2002)
- The Radiographic Atlas of Skeletal Development of the Foot and Ankle (Hoerr *et al.*, 1962)

These studies will be discussed in detail in sections 3.1 and 3.2 respectively.

2.3 Study phase 2 – materials and methods

2.3.1 Study sample

Prior to the commencement of this study, permission was sought from, and granted by, the Information Governance Officer of Tayside NHS for access to and use of clinical radiographic images for use in this research (see appendix C). The Tayside NHS Trust supplies healthcare to approximately 386,600 patients through 18 hospitals in three local authority areas (The Scottish Government, 2010). As Tayside NHS utilises a common radiographic database for all hospitals administrated by the health board, individuals included in this study may have attended a hospital other than Ninewells and have been resident in a local authority other than Dundee City.

All radiographs included in this study were taken as a result of normal clinical practice following attendance at an Accident and Emergency department or as part of clinical monitoring of a previous injury between 2008 and 2011. Initially, images were viewed and downloaded directly from the Picture Archiving and Communication System (PACS) database in Joint Photographic Experts Group (JPEG) format from a terminal within the radiology department of Ninewells Hospital.

The data collected for each individual included:

- Date of birth
- Date of image acquisition
- Side of the body represented in the image

- Biological sex
- Radiographic plane in which the image was obtained

Images included in this study were obtained from adult females and males between 20 and 50 years of age inclusive and represented both sides of the body. Chronological age of the individuals included within the study was calculated as the difference between their recorded DOB and the DOI. To simplify any future analyses, the calculated chronological age was rounded to the number of completed months. To prevent duplication of results, images of the left and right sides of the body were obtained from separate individuals. Similarly, two separate image sets were collected for the distal femur and proximal tibia to ensure that no duplication of individuals occurred.

Where possible, five images were obtained for each age group for left and right sides resulting in ten images per year group in both female and male cohorts. This was occasionally limited by the availability of images that satisfied the inclusion criteria. Although care was taken during initial vetting of images to exclude those in which the region of interest was obscured, some images were missed and subsequently rejected during the analysis phase of the study.

To ensure anonymity, images were assigned a URN which included information relating to the sex, side of the body and anatomical region from which the image was obtained and a sequential number, for example MLDT1 corresponded to male left distal tibia number 1. Radiographs were collected from five anatomical regions: proximal humerus, distal radius, distal femur, proximal tibia and distal tibia and had been taken in the anterior-posterior (A-P) plane. Where available, radiographs taken in the medial-lateral (M-L) plane were also obtained. In such cases, a sequential letter was added to the URN of the individual, for example MLDT1a. These anatomical areas were selected due to their common usage in skeletal age estimation and the availability of appropriate radiographic images on which consistent examinations of epiphyseal scars could be made. This included a relatively consistent orientation of the joint when the radiographs were taken.

Due to the nature of the images used in this study and the ethical requirement of patient confidentiality, it was not possible to collect information relating to the

ancestral origin of the individuals or any previous or current medical conditions not specifically noted by the radiologist. In cases where a previous condition or injury which may have affected the growth plate was noted by the radiologist, the individual was excluded from the study. This included but was not limited to:

- Hip dysplasia.
- Precocious or delayed puberty.
- Varus or valgus deformity at the knee or ankle.
- Previous or current fracture involving the region of interest.
- Chronic illness e.g. diabetes, cancer

The number of radiographs obtained for inclusion in the study is presented in Table 2.1 according to anatomical region, biological sex and the side of the body represented by the image.

Table 2.1: Phase 2 sample distribution according to region, sex and side of the body

Region	Female Left	Female Right	Male Left	Male Right	Total
Prox. Humerus	155	155	154	155	**619**
Distal Radius	155	153	153	155	**616**
Distal Femur	135	137	150	139	**561**
Prox. Tibia	155	153	154	155	**617**
Distal Tibia	152	150	149	149	**600**
Total	**752**	**748**	**760**	**753**	**3013**

2.3.2 Method

2.3.2.1 Image analysis method

Using Adobe Photoshop™, each radiograph was copied, cropped to include the region approximate to the location of the former growth plate and marked with a grid to demarcate six tracks spanning from the medial to the lateral extremities of the bone when viewed in the A-P plane. The division of each radiograph into six equally spaced tracks normalised the data and thereby facilitated the comparison of the level of persistence or obliteration of the epiphyseal scar between individual tracks as well as between regions. This number of tracks was considered to be optimal in terms of the precision of the analysis and also reducing the effect of intra-observer ambiguity in the interpretation of the epiphyseal scar.

All analyses, with the exception of the distal femur, were conducted in the A-P plane. Due to the effect of superimposition of the patella, analysis of the distal femur was carried out in the M-L plane. The positions of the tracks in each anatomical area are presented in Figures 2.4 to 2.8. Where obtained in the A-P plane, track 1 was placed in the most medial and track 6 in the most lateral aspects of the bone. In the case of the distal femur, track 1 was located in the most anterior and track 6 in the most posterior regions of the bone. This facilitated a direct comparison between left and right sides of the body without the requirement for mirroring the image.

Figure 2.4: Placement of distal radius assessment tracks

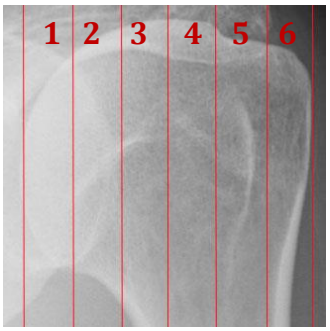

Figure 2.5: Placement of proximal humerus assessment tracks

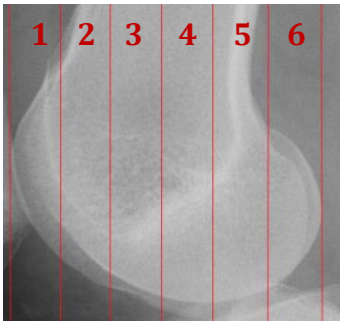

Figure 2.6: Placement of distal femur assessment tracks

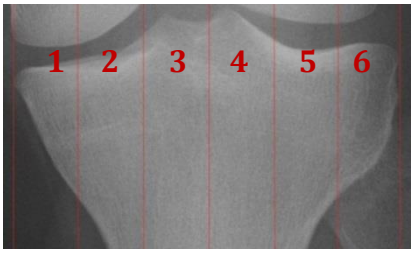

Figure 2.7: Placement of proximal tibia assessment tracks

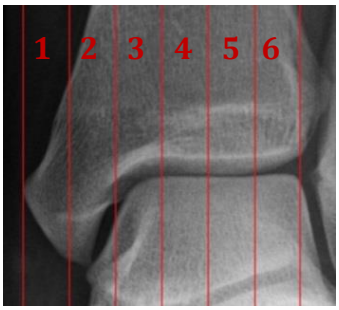

Figure 2.8: Placement of distal tibia assessment tracks

Within each of the six tracks, the presence of the epiphyseal scar was quantified using a scoring system according to the criteria presented in Table 2.2.

Table 2.2: Scoring criteria for the epiphyseal scar

Score	Criteria
0	No epiphyseal scar observed within the track
1	A partial or fenestrated scar observed within the track
2	Epiphyseal scar completely traverses the track
X	No assessable bone present within the track

From these data, the Total Persistence Score (TPS) was calculated for each individual as the sum of the scores assigned to each of the six tracks. This formed the data set from which all subsequent analyses would be undertaken. In addition to TPS, the sum of the scores assigned to tracks 1 and 2, 3 and 4 and 5 and 6 were calculated for the medial, central and lateral areas of the epiphyseal scar as respectively. These scores are termed the Regional Persistence Score (RPS).

2.3.2.2 Data handling and statistical analysis

Within each sex-specific and side specific group, the TPS values calculated for each individual were recorded against the chronological age, sex and side of the body from which the image was taken. Initial analysis was undertaken to determine the percentage of individuals within each sex and anatomical region for whom some

remnant of the epiphyseal scar was observed. This is termed the Total Persistence Rate (TPR). Subsequent statistical assessments were undertaken and included Shapiro-Wilk Normality tests, one-way ANOVA and General Linear Model (GLM) analyses. All initial data handling was conducted using Microsoft Excel™ and all statistical tests were undertaken using either Sigmaplot 12.0™ or IBM SPSS™ statistics software. The statistical analyses of regional persistence scores were conducted in the same manner as those of TPS.

2.3.2.3 Intra-observer and inter-observer assessment

Analyses of intra-observer and inter-observer consistency were undertaken for each anatomical region using a sample of 60 randomly selected radiographs. As the level of variation in development between the left and right sides of the body in multiple skeletal areas has been reported to be insignificant, only left sided radiographs were included in the intra-observer and inter-observer samples (Dreizen *et al.*, 1957; Loder *et al.*, 1993; Cheng *et al.*, 1998). For each anatomical region, radiographs from 30 females and 30 males were assessed according to the method outlined in section 2.3.2.1 of this chapter.

Intra-observer assessment

Intra-observer analyses for each region were conducted following the completion of all assessments on the main study sample, at which time no statistical analyses had been undertaken. The chronological ages of the individuals included in the intra-observer subsample were not revealed to the observers. Following the completion of the assessments, statistical analysis of the data was undertaken initially through the calculation of sample distribution according to TPS and percentage agreement and subsequently through the use of one-way ANOVA and GLM analyses. For the purposes of this study and to include the potential for minor variation in the assignment of TPS which may result from variation in the resolution of the monitor on which the images were assessed, percentage agreement was interpreted as absolute agreement ±2 scores.

Inter-observer assessment

Inter-observer analyses for each region were undertaken by three separate observers, each of whom holds a PhD. in anatomy, forensic anthropology or human

identification. These observers represented various levels of experience in radiographic interpretation and were ranked according to their level of experience with the least experienced observer labelled “observer 1” and the most experienced observer labelled “observer 3”. “Observer 2” was considered to have an intermediate level of experience. All inter-observer assessments were conducted using the same samples of radiographs collated for use in the intra-observer analyses. To standardise the position of tracks within the images, all sample radiographs were marked with tracks prior to their distribution. To facilitate an analysis of the effect of experience on inter-observer consistency, the observers were instructed in which order to complete their assessments (females first). This protocol was followed for all anatomical regions. The data resulting from the inter-observer analyses were assessed in the same manner at those resulting from the intra-observer analyses.

Study Phase 1 – Skeletal age estimation from the juvenile foot and ankle

The following section of this thesis represents the work conducted during the first year of this study as a Master of Science by Research under Ordinance 12 (research students) of the Charter of the University of Dundee.

3 Age Estimation from the Foot and Ankle: A Test of Two Methods

The importance of the foot and ankle in forensic case work has been highlighted in recent years as the number of incidents in which a foot has been recovered has increased. This has been accompanied by a rise in the media attention surrounding such cases (British Broadcasting Corporation, 2004; 2007; 2008a; 2008b; 2008c; Gunn, 2008; British Broadcasting Corporation, 2009; 2010). In the UK, the discovery of isolated lower limbs or feet most frequently occurs in the vicinity of coastal areas or estuaries, where, due to fluvial or marine action, remains are deposited at the high water mark (British Broadcasting Corporation, 2004; 2007; 2009; 2010). Since the year 2000, there have been at least eight incidents in the UK where human remains have been recovered, consisting of only legs or feet, including two in 2010 and three in 2008. Elsewhere, nine feet were recovered on the pacific north-west coast of north America in a 27 month period (British Broadcasting Corporation, 2008b; 2008c; Gunn, 2008). In all of these cases, the foot was contained within an article of footwear, which likely afforded a degree of protection to the remains from environmental factors and prevented disarticulation of the foot (Haglund and Sorg, 2002).

To ascertain why the foot is more commonly recovered than other body parts it is first necessary to understand the order in which the body disarticulates as part of normal decomposition. The disarticulation sequence is included in the division of forensic anthropology known as forensic taphonomy. Forensic taphonomy is the study of the postmortem processes to which the body is subjected and are dependent on the environment in which the remains are located (Haglund and Sorg, 1996). In terrestrial systems, as in marine environments, the remains will

become likely integrated into the food chain. As a result of scavenging, the distal limb segments are often among the first to be disarticulated from the core mass (Haglund *et al.*, 1989; Haglund and Sorg, 2002). In marine and fluvial environments, water movement also affects the rate of decomposition and disarticulation (Haglund and Sorg, 2002). Subsequent decomposition of body tissues results in the detachment of osseous elements from the mass assemblage. In the case of the upper limb, this could result in loss of the distal portion of the limb, while the proximal portion may be retained by clothing. In addition to increasing the likelihood of retention of elements, footwear also offers the disarticulated remains a degree of buoyancy for cases where bodies of water are thought or known to be involved, thereby positing a potential explanation for the high incidence of feet recovered in isolation.

The establishment of a positive identification in cases where only a small proportion of remains is recovered may be problematic if there is no DNA match. In such cases, it is the responsibility of the forensic anthropologist to provide the investigating officers with as much information as possible relating to the four parameters of biological identity i.e. sex, age, ancestry and stature (Scheuer, 2002). Studies have been undertaken to examine the relationship between stature and metatarsal length (Byers *et al.*, 1989); stature and height of the calcaneus and talus (Holland, 1995); and the relationship between overall size of the foot, stature and biological sex (Ozden *et al.*, 2005; Krishan and Sharma, 2007). There is however, a distinct paucity of literature surrounding the use of the foot in skeletal age estimation, with only two approaches being published in the past five decades (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002).

To satisfy the requirements of repeatability and reliability, approaches utilised in skeletal age estimation must be exposed to repeated testing and continuous scrutiny to maintain their accuracy when applied to current population groups (The Law Commission, 2011). Without adequate testing and, if appropriate, revision, estimations of skeletal age based on the foot and ankle may be less reliable than those from other anatomical regions that have been subject to scrutiny.

3.1 A Test of the Scoring System for Estimating Age in the Foot Skeleton

3.1.1 The Whitaker method (2002)

The method devised by Whitaker and colleagues (2002) is a complex, multi-stage scoring system, whereby independent maturity scores are assigned to each of sixteen bones within the foot. These include the calcaneus, metatarsals and proximal and distal phalangeal row. Initially, an ossification score is assigned to each primary and secondary centre of ossification included in the method, according to the criteria outlined in Table 3.1.

Table 3.1: Criteria for assignment of ossification scores for primary and secondary centres. Adapted from Whitaker *et al*. (2002)

Score	Criteria for Primary and Secondary Centres of Ossification
0	No bony material visible on x-ray due to artefact or film quality
1	Ossification centre absent
2	Ossification centre present but rudimentary
3	Ossification centre present and mineralised bone resembles adult shape
4	A fully adult bone (assumes complete fusion)

Following this, a score is assigned to the degree of fusion observed between the primary and secondary centres according to the criteria presented in Table 3.2. The fusion score is then combined with those assigned to the primary and secondary centres of ossification to provide a three-digit sequence which corresponds to a maturity score as presented in Table 3.3.

Table 3.2: Criteria for assignment of fusion scores. Adapted from Whitaker *et al.* (2002)

Score	Criteria for Fusion of Ossification Centres
0	Bones obscured or secondary centre unformed
1	Primary and secondary centres far apart
2	Primary and secondary centres approximate one another but still separated by radiolucent band
3	Primary and secondary centres in partial fusion; radio-opaque connections present with radiolucent spaces
4	Primary and secondary centres in full contact; no radiolucent spaces but epiphyseal line still apparent
5	Fully adult fusion; no epiphyseal line apparent

Table 3.3: Ossification and fusion scores and their corresponding maturity code. Adapted from Whitaker *et al*. (2002)

Primary Score	Secondary Score	Fusion Score	Maturity Code
0	0	0	X
1	1	0	X
2	1	0	1
2	2	1	2
3	1	0	3
3	2	1	4
3	2	2	5
3	3	1	6
3	3	2	7
3	3	3	8
3	3	4	9
4	4	5	10

The maturity score assigned to each bone is then cross-matched with a final table (Table 3.4), from which a corresponding sex-specific age range for each bone is assigned. A final estimated chronological is then calculated from the highest younger estimated age and the lowest older estimated age assigned to the individual.

Table 3.4: Estimated age ranges in months according to maturity code, bone and sex. Adapted from Whitaker *et al*. (2002)

Bone	Maturity Code							
	1	**3**	**4**	**5**	**7**	**8**	**9**	**10**
Cal.	31-36	42-114	76-113	100-140	117-159	131-183	144-194	175-213
	5-48	37-95	62-104	83	97-125	106-153	118-163	149-219
MT 1			36-63	67-114	56-141	95-174	106-204	203-226
			40-86	32-84	37-152	84-163	118-163	149-219
MT 2	3-31	63	36-56	67-76	83-183	100-174	163-194	203-226
	13	5-32	39-86	37-84	57-154	84-163	118-160	149-219
MT 3	3-31	63	36-56	67-76	83-183	106-174	163-194	203-226
	13	5-86	39-117	37-84	57-154	101-163	127-160	149-219
MT 4	3-31	36-63	42-114	37-93	83-183	106-174	163-194	203-226
	13	5-86	37-117	84	57-154	97-163	132-160	149-219
MT5	3-31	36-63	42-114	67-76	83-183	106-174	163-194	203-226
	13	5-86	37-117	62-84	84-154	97-163	132-160	149-219
PP1			36-114	76-112	56-183	95-194	163-204	203-226
			39-86	48-84	37-154	39-163	118-216	149-219
PP 2	3-31		36-63	56-183	56-183	95-165	156-194	203-226
			32-86	37-84	39-152	118-202	118-163	149-219
PP 3	3-31		36-63	76-131	56-183	67-165	159-194	203-226
	5		32-86	37-84	39-152	101-163	118-202	149-219
PP 4	3-31		36-63	76-131	56-183	95-165	159-194	203-226
	5		32-86	37-84	39-152	101-163	118-202	149-219
PP 5	3-31		36-50	76-131	56-183	67-165	154-204	203-226
	5		32-86	37-84	49-152	101-163	118-202	149-219
DP 1			36-63		56-183	37-163	155-194	203-226
			39-86		37-125	84-163	118-202	141-219
DP 2	3-63	50-114		93	67-165	144-155	150-204	159-226
	40	32-86	39-60	83	49-125	95-163	150-163	118-219
DP 3	3-63	50-114	108	93	67-165	144-155	150-194	159-226
	39	32-86	39-60	83	49-125	101-163	97-163	118-219
DP 4	3-63	50-114	108	93	67-165	144-159	163-194	159-226
		37-86	50-60	83	62-121	97-163	126-163	106-219
DP 5	31-131	50-113		111	67-165	93-150		107-226
	40-77	39-121		100	84-119	97-129	126-163	62-219

Cal = Calcaneus. MT= Metatarsal. PP=Proximal Phalanx. DP=Distal Phalanx

For each bone and maturity code, males are represented by the top row of data, females by the second row of data; absent estimated ages are represented by blacked out cells.

3.1.1.1 Example of the application of the Whitaker (2002) method

Due to the complexity of the Whitaker method, an example of its application is presented in the following section with reference to the radiograph presented in Figure 3.1.

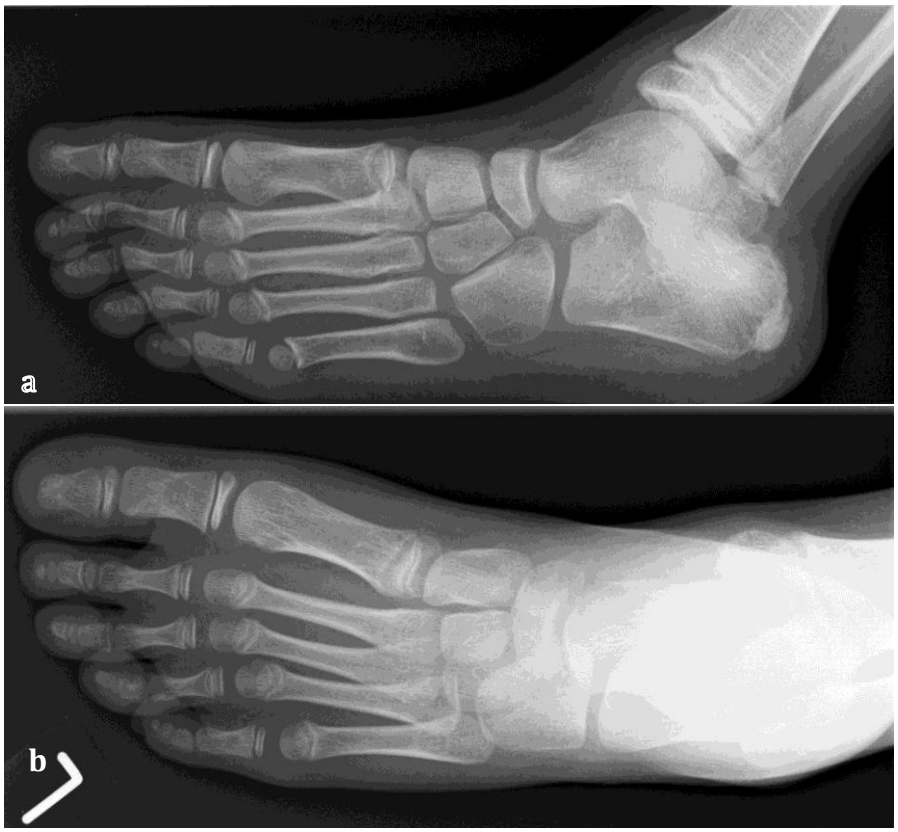

Figure 3.1: Radiograph of the left foot of a female, aged 7 years 9 months, in (a) M-L view and (b) A-P view

As the Whitaker method requires evaluation of the primary and secondary centres of ossification for sixteen skeletal elements, this illustration of the method will be restricted to a single bone. In this case, this shall be the proximal phalanx of the first pedal ray (Figure 3.2).

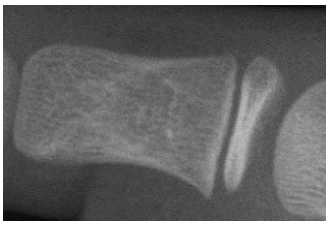

Figure 3.2: Proximal phalanx of first pedal ray

According to the criteria established by Whitaker *et al*. (2002), the bone presented in Figure 3.2 would be assigned the values of 3, 2 and 2 for the ossification of the primary and secondary centres and the degree of fusion between them. This corresponds to a maturity score of 5 and the associated estimated age range of between 76 and 112 months. This process is repeated for all sixteen skeletal elements included in the method.

3.1.1.2 Demographics of the sample used in the development of the Whitaker et al. (2002) method

The Whitaker (2002) method for estimating age from the bones of the foot was developed from a cross-sectional sample of radiographs from podiatric clinics in San Francisco Bay, California. This study was based on radiographs from 143 individuals, consisting of 73 males and 70 females. Subjects included in the sample were aged between birth and 20 years of age, although not all age cohorts were represented by an equal number of individuals. Consequently, the original method was only tested on the most highly populated age groups, between 8 and 14 years of age (Whitaker *et al.*, 2002).

Although not presented in the original publication, to account for population variation in skeletal development as a result of resource acquisition it is necessary to consider the demographic characteristics of the population area from which the study sample was drawn (Schmeling *et al.*, 2000). According to the 2010 census, the population of San Francisco, California, and the surrounding boroughs consists of a population of approximately 7,150,739 individuals, 50.4% of whom are female (Metropolitan Transportation Commission and Association of Bay Area Governments, 2010). The ancestral diversity of the population of San Francisco Bay is complex and is presented in Table 3.5.

Table 3.5: Ancestral diversity of the San Francisco Bay region in 2010 (Metropolitan Transportation Commission and Association of Bay Area Governments, 2010).

Ancestry	Percentage of population
White	52.5
Asian	23.3
African American	6.7
First Nations †	1.3
Other	10.8
Two or more	5.4

† includes Amerindian, Inuit, Hawaiian and Pacific Islanders

Data on levels of income and poverty in 1999 suggest that the median household income of the population was $75,989 *per anum* and median income was $38,294 *per capita* (Metropolitan Transportation Commission and Association of Bay Area Governments, 2010). Within the population of the San Francisco Bay area, 9.7% of individuals are considered to be in poverty (Metropolitan Transportation Commission and Association of Bay Area Governments, 2010); compared with the California state mean of 14.4% and the national mean of 14.3% (United States Census Bureau, 2011); however no precise definition of poverty is given.

3.1.2 Method for testing the scoring system for estimating age in the foot skeleton (Whitaker et al., 2002)

Despite nearly a decade having passed since the initial publication of this method, it remains the most recent attempt at age estimation from the bones of the foot and ankle. This method has not been tested against any sample other than that from which it was originally derived. All analyses were therefore conducted in the manner set-out in the original publication.

3.1.2.1 Sample used in the test of the Whitaker (2002) method

As not all maturity scores correspond to an age range for all bones presented in the Whitaker method (2002) (see Table 3.4), the sample used in this analysis was restricted to radiographs in which an unobstructed view of all the relevant centres of ossification was presented. Consequently, radiographs that did not include a clear view of all the required centres of ossification or the region in which they would appear were omitted from the sample used in the test of the Whitaker method. This reduced the number of radiographs suitable for use in the study to 260 individuals (139 males and 121 females). The distribution of this sample according to sex and age is presented in Figure 3.3.

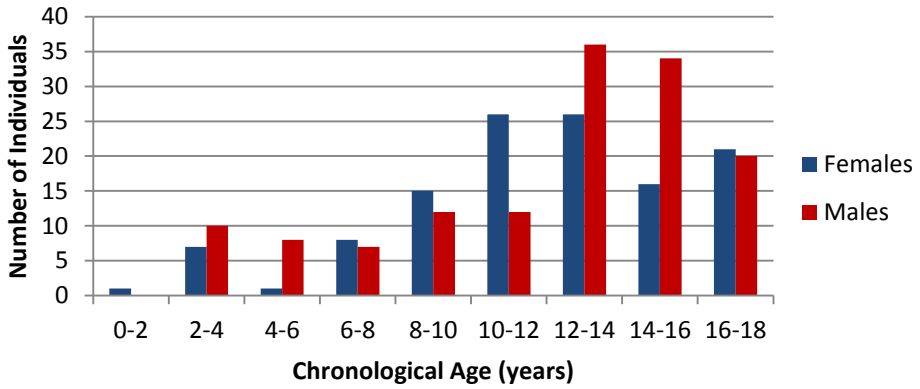

Figure 3.3: Distribution of the Whitaker *et al.* (2002) study sample according to sex and chronological age

3.1.2.2 Analysis of the Whitaker (2002) method

Composite age ranges were constructed according to the method outlined by Whitaker *et al*. (2002). All analyses were conducted without cognisance of the chronological ages of the individuals. The accuracy of the composite age ranges was tested through the comparison of the estimated age range with the known chronological ages of the individuals in the sample. The percentage of individuals for whom the estimated age range included the chronological age was calculated for each 2 year cohort. In addition, the precision of the method was assessed through the calculation of the maximum and minimum age ranges within each 2 year cohort.

To adapt the method of Whitaker *et al.* (2002) to conform to forensic principles as outlined by Ritz-Timme *et al*., (2000), a second set of composite age ranges was constructed using the youngest and oldest ages from the ranges calculated for each of the skeletal elements considered by the source method. The accuracy and precision of the adjusted composite age ranges were assessed in the same manner as the original composite age ranges.

The statistical analysis for this study was carried out using Sigmaplot 12™ and SPSS statistics software.

3.1.2.3 Intra- and inter-observer analysis method

Intra- and inter-observer variation were assessed using a randomly selected subset comprising radiographs from 30 female and 30 male left feet. Due to the

complications imposed through the provision of age ranges rather than a single estimated age bounded by a standard deviation, intra- and inter-observer variation was calculated according to the assignment of maturity score.

The percentage agreement between the maturity scores assigned to the sixteen bones for each individual was calculated. The second round of analysis was carried out by the author in excess of four months after the first round and was completed without knowledge of the chronological ages of the individuals in the subset or the maturity scores assigned on the first pass.

The sample of images collated for use in the intra-observer analysis was assessed by an additional observer to determine the consistency of the method between examiners. The percentage agreement between the maturity scores assigned to the sixteen bones for each individual was calculated. The assessments were conducted by the second observer without knowledge of the maturity scores assigned by the first observer or the chronological ages of the individuals within the sample.

3.1.3 Results

3.1.3.1 Results of the intra- and inter- observer analysis

Intra-observer analysis

The percentage intra-observer agreement and degrees of divergence are presented in Figure 3.4.

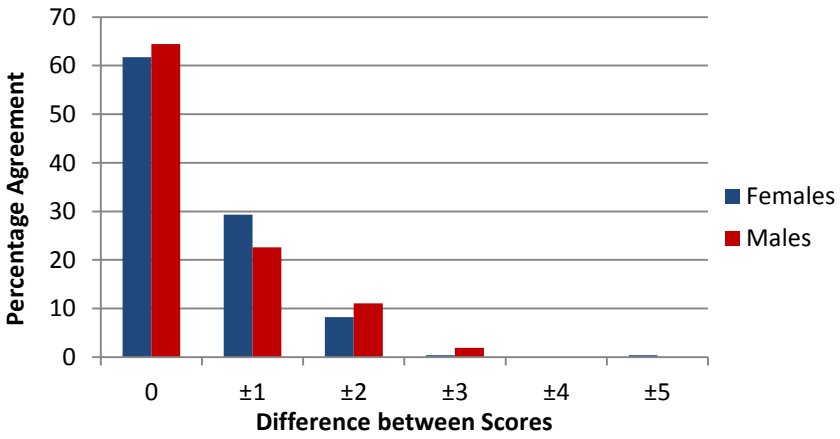

Figure 3.4: Percentage agreement achieved during intra-observer analysis

In female individuals, intra-observer agreement between the maturity scores assigned on two occasions occurred in 61.72% of the bones examined. Further analysis of the discordant data showed that 29.3% of assessments disagreed by a single score, 8.2% of assessments varied by 2 scores, and 0.39% disagreed by 3 scores. Intra-observer variation that exceeded 3 maturity scores was only observed in a single bone. Although variation was observed in the assignment of maturity scores, the result of a one-way analysis of variance (ANOVA) on ranks test showed that there was no statistically significant variation between the maturity scores assigned during the first and second rounds of assessment (P=0.248; H=1.333).

Analysis of the data derived from the examination of radiographs from the male sample showed that intra-observer agreement occurred in 64.42% individuals. Examination of the conflicting intra-observer data showed that 22.6% of assessments varied by a single score and 11.06% differed by 2 scores. The remaining 1.92% varied by 3 scores. The result of a one-way ANOVA on ranks suggested that there was no statistically significant variation between the maturity scores assigned to male individuals during the first and second rounds of assessment (P=0.933; H=0.000713).

Inter-observer analysis

The results of the inter-observer analyses according to percentage agreement and disagreement are presented in Figure 3.5.

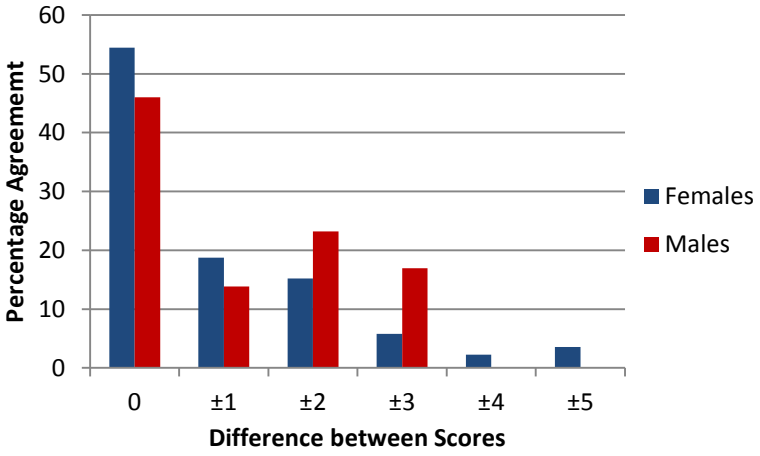

Figure 3.5: Percentage agreement achieved during inter-observer analysis of the Whitaker *et al.* (2002) method

In the female sample, inter-observer agreement in the assignment of maturity scores occurred in 54.46% of bones. Further examination of the discordant data showed that 18.75% of assessments varied by a single score, 15.18% differed by 2 scores and 5.8% by 3 scores. The remaining inter-observer conflicts (5.8%) of assessments varied between 4 and 5 scores. The result of a one-way ANOVA showed that inter-observer variation in the assignment of maturity scores was statistically significant (P=0.013; H=6.123).

In the male sample, inter-observer agreement in the assignment of maturity scores occurred in 45.98% of bones. Analysis of the discordant data showed that 13.84% of assessments differed by a single score, 23.21% varied by 2 scores and 16.96% by 3 scores. The result of a one-way ANOVA suggested that the variation observed in the assignment of maturity scores within the male sample was statistically significant (P<0.001; H=20.284).

3.1.3.2 Results of the test of the Whitaker (2002) method

Females

As the Whitaker *et al*. (2002) method provides estimated age ranges, it was necessary to first establish the percentage of the sample whose chronological age fell within the age range estimated by the method. In addition , the mean spans of the age ranges assigned to individuals of each two year cohort were calculated. The results of these calculations, together with the maximum and minimum span of the composite age ranges generated within each two year age cohort, are presented in Table 3.6.

Table 3.6: Percentage inclusion of chronological age within Whitaker composite age ranges for females

Age Cohort (years) (n=121)	Percentage Inclusion of Chronological Age	Mean Range Span (months)	Max Range Span (months)	Min Range Span (months)
0-2 (n=1)	0	8	8	8
2-4 (n=7)	0	30.29	46	12
4-6 (n=1)	100	21	21	21
6-8 (n=8)	12.5	21	26	0
8-10 (n=16)	40	17.47	51	2
10-12 (n=25)	30.8	17.31	53	2
12-14 (n=26)	57.7	36.69	73	2
14-16 (n=16)	56.25	45.44	70	11
16-18 (n=21)	100	70	70	70

Although initial analysis showed that only 50.4% of chronological ages fell within the estimated age range, the overall trend observed in the percentage inclusion of chronological age within the composite estimated age ranges suggests that older individuals are more likely to be represented by the age range assigned according to the method devised by Whitaker *et al*. (2002). Further examination of the results showed that the trend observed in increasing percentage inclusion was mirrored by an increase in the maximum span of the final composite age range. In this sample of radiographs from female individuals, the maximum span of the Whitaker composite age ranges was 73 months, corresponding to 6 years and 1. This was observed in individuals between 12 and 14 years of age. The minimum age range assigned during this analysis was observed in both cohorts between birth and 4 years of age. Within these cohorts, no individuals were represented by the estimated age ranges derived from the application of the Whitaker (2002) method.

Analysis of the composite age ranges suggested that the Whitaker *et al*. (2002) method is more likely to assign a composite age range which is inclusive of the chronological age to individuals over the age of 12 years than to individuals between birth and 12 years. Only two cohorts were shown to exhibit complete inclusion of chronological age within the estimated age range; however one of these was represented by a single individual and therefore should not be considered a reliable predictor of application of the method to that age group (4-6 years).

To adapt the method of Whitaker *et al*. (2002) to conform to the forensic principles outlined by Ritz-Timme *et al*. (2000), a second set of composite age ranges was constructed using the youngest and oldest ages from the ranges calculated for each of the skeletal elements considered by the source method. This created an additional series of composite age ranges which represented all possible ages based on the stage of skeletal development observed in each individual. These ranges are termed the **adjusted** composite age ranges. The percentage of individuals whose chronological age was included within the adjusted composite age ranges as well as the mean, maximum and minimum numbers of months represented by the **adjusted** composite age ranges were presented in Table 3.7.

Table 3.7: Percentage inclusion of chronological age within adjusted composite age ranges for females

Age Cohort (years) (n=121)	Percentage Inclusion of Chronological Age	Mean Range Span (months)	Max Range Span (months)	Min Range Span (months)
0-2 (n=1)	100	116	116	116
2-4 (n=7)	71.4	115.4	126	88
4-6 (n=1)	100	147	147	147
6-8 (n=8)	100	133.8	182	117
8-10 (n=16)	100	156.6	182	126
10-12 (n=25)	100	166.2	182	126
12-14 (n=26)	100	164.5	182	126
14-16 (n=16)	100	158.4	180	157
16-18 (n=21)	100	157	157	157

When the results were considered across the entire sample, the chronological ages of 98.4% of individuals were included in the estimated age ranges. Although a marked increase in the percentage of individuals whose chronological age was included in the estimated age range was observed, this was accompanied by a dramatic rise in the number of months included in the maximum and minimum age ranges for each 2 year cohort. The maximum span of the **adjusted** composite age ranges for the female sample was 182 months. This was observed in all cohorts between 6 and 14 years of age. The minimum span of an estimated age range was 88 months. This age range, corresponding to 7 years and 4 months, was observed in individuals represented by the 2-4 year age cohort.

Males

The percentage of male individuals whose chronological age was included in the composite estimated age range and the maximum and minimum age ranges assigned to individuals within each 2 year cohort are presented in Table 3.8.

Initial analysis of these results showed that the total percentage of individuals whose chronological age fell within the assigned range was 21.6%. The overall trend observed in the percentage inclusion of chronological age within the estimated age range suggested that older individuals may be more likely to be represented by the estimated age range than younger individuals. As no subjects were included in the birth to 2 year cohort, no data is available for the analysis of the Whitaker method in this age group.

Table 3.8: Percentage inclusion of chronological age within Whitaker composite age ranges for males

Age Cohort (years) (n=139)	Percentage Inclusion of Chronological Age	Mean Range Span (months)	Max Range Span (months)	Min Range Span (months)
0-2 (n=0)	-	-	-	-
2-4 (n=12)	20	12.9	37	6
4-6 (n=7)	20	21.9	37	6
6-8 (n=7)	14.3	11.3	20	9
8-10 (n=12)	16.7	8.0	40	2
10-12 (n=12)	8.3	6.1	21	2
12-14 (n=35)	36.1	7.6	24	2
14-16 (n=34)	5.9	10.1	23	1
16-18 (n=20)	35	9.7	10	1

The results also indicate that individuals between 2 and 8 years of age were likely to be represented by wider age ranges than those aged 8 years or over. The maximum estimated age range observed in the male sample of 40 months was recorded in the 8-10 year cohort. The minimum range of 1 month was recorded in both 2 year cohorts between 14 and 18 years of age.

Currently accepted principles of age estimation require an age range to be of sufficient inclusivity to accommodate all possible ages for which there is osteological evidence while maintaining exclusivity in order to isolate any erroneous data which cannot be supported by scientific evidence (Ritz-Timme *et al.*, 2000; Rösing *et al.*, 2007). Table 3.9 presents the percentage inclusion of the chronological age within the **adjusted** composite age ranges.

These results showed that the **adjusted** age ranges included the chronological age of 98.6% of the sample. The only cohort in which the chronological age was not included in the estimated age range in every subject was that including individuals between 2 and 4 years of age. Although these results suggest that the adjusted composite age ranges are highly inclusive, the excessive widths of the age ranges suggest that they are of virtually no practical value. The minimum span of an estimated age range was 78 months (6 years, 6 months). This was observed in both cohorts between 2 and 6 years of age. The maximum span of an estimated age range was 189 months (15 years, 9 months). This was observed in the 14-16 year age cohort.

Table 3.9: Percentage inclusion of chronological age within adjusted composite age ranges for males

Age Cohort (years) (n=139)	Percentage Inclusion of Chronological Age	Mean Range Span (months)	Max Range Span (months)	Min Range Span (months)
0-2 (n=0)	-	-	-	-
2-4 (n=12)	18.2	93.7	147	78
4-6 (n=7)	100	105.8	147	78
6-8 (n=7)	100	79.3	168	141
8-10 (n=12)	100	141.3	170	92
10-12 (n=12)	100	142.5	170	127
12-14 (n=35)	100	154.0	170	127
14-16 (n=34)	100	145.1	189	119
16-18 (n=20)	100	121.1	159	119

Although the chronological age of a high percentage of male individuals fell within their assigned age range, the adjusted composite age ranges are prohibitively wide and therefore of little practical value within the context of forensic age estimation.

3.1.4 Discussion of the Whitaker et al. (2002) approach to age estimation

3.1.4.1 Discussion of the intra-observer and inter-observer analysis of the Whitaker (2002) method

To satisfy the requirements of reliability and repeatability, the Whitaker *et al.* (2002) method was tested against a sample of radiographs of the foot from juvenile individuals of a known chronological age, on multiple occasions and by multiple observers. This study found that intra-observer agreement was likely to occur on 61.72% and 64.42% of occasions in the female and male sample groups respectively. Although this suggests that a high degree of intra-observer error may occur, the variation between assessments was not found to be statistically significant. In contrast to the findings of this study, no intra-observer error was reported to have occurred in the original study of Whitaker *et al*. (2002). The discrepancy between the levels of intra-observer variation reported in this study and those of Whitaker *et al*. (2002) may be related to the number of individuals included in the intra-observer assessment, as inclusion of a larger sample size may lower the risk of recalling the score assigned on previous assessments and thereby increase the level of intra-observer disagreement.

To be considered suitable for application, methods of forensic assessment must present consistent results when applied by multiple observers. The results of this study pertaining to the level of inter-observer error found that 45.54% and 54.02% of assessments conducted on females and males respectively disagreed. In contrast to the results obtained through the analysis of intra-observer error, the variation in the maturity scores assigned during the inter-observer assessment phase were found to be statistically significant in both sex cohorts. These findings indicate that some degree of inter-observer variation in the assignment of maturity scores is likely to occur. The high degree of inter-observer disagreement observed in this analysis is in contrast to that published within the original method where error rates of between 0 and 30% were recorded. The low level of intra-observer and inter-observer agreement in the assignment of maturity scores in the analysis of the Whitaker method (2002) may be attributable to the high degree of complexity of the method and the level of subjective interpretation of the criteria to which each ossification and fusion score corresponds.

While the data presented in the original publication may suggest that the application of the method is likely to yield consistent results, the findings of this study suggest that the Whitaker *et al*. method (2002) does not meet the criteria of reliability and repeatability that are expected of methods of forensic assessment within the judicial systems of the UK (The Law Commission, 2011).

3.1.4.2 Discussion of the analysis of the Whitaker (2002) method

Prior to the submission of the results of new techniques of age estimation as evidence in court, it is good practice for them to be subject to validation studies carried out on populations other than that on which the method was derived (The Law Commission, 2011). The Whitaker scoring system for estimating age from the bones of the foot (2002) is the most recently produced method of age estimation from this skeletal region. This study represents the first attempt to determine the accuracy and reliability of this method as a tool in forensic age estimation.

This study found that the estimated age ranges generated through the application of the Whitaker method (2002) included the chronological age of female individuals on 50.4% of occasions. This was reduced to 21.6% of occasions for

male individuals. It is suggested that the observed discrepancy in percentage inclusion between females and males may be related to the relative width of the age ranges assigned to each sex. Through examination of the data relating to the mean width of the Whitaker (2002) composite age ranges assigned within each 2 year age cohort, it was noted that those attributed to the male sample were significantly smaller than those observed in the female sample. This may account for the relative difference in the percentage inclusion of chronological ages within the estimated age ranges as derived from the application of the original Whitaker method (2002).

The low percentage inclusion of the chronological ages within the estimated age ranges may be partially attributable to the manner by which the age ranges were constructed. The use of the oldest younger age range and younger oldest age range produces an overall estimated age range which is artificially narrowed. This was evidenced by the findings of this study which showed that in some cases, the oldest younger age and youngest older age were equivalent and therefore no age range was established. This therefore contradicts the recommendation that an estimation of age should always be accompanied by a range of variation (Workshop of European Anthropologists, 1980; Ritz-Timme *et al.*, 2000; Rösing *et al.*, 2007).

To rectify the discrepancy between the methodology of Whitaker *et al.* (2002) and the recommendations for forensic age estimation, an adjustment was made to the manner with which the final estimated age ranges were constructed (Ritz-Timme *et al.*, 2000). This concerned the alteration from the published method to the construction of age ranges from the youngest and oldest potential chronological age based on the age ranges assigned to each bone examined by the method. This change in approach resulted in an increase in the percentage inclusion of the chronological age within the estimated age range to 98.4% and 98.6% for females and males respectively. Although the manner in which the adjusted composite age ranges were constructed is in accordance with good practice for forensic age estimation (Workshop of European Anthropologists, 1980; Ritz-Timme *et al.*, 2000); the maximum and minimum spans of the age ranges within each cohort are unacceptably wide, resulting in age ranges of virtually no practical value.

3.2 A Test of the Radiographic Atlas of Skeletal Development of the Foot and Ankle

3.2.1 The Radiographic Atlas of Skeletal Development of the Foot and Ankle (Hoerr et al., 1962)

The radiographic atlas of skeletal development of the foot and ankle (Hoerr *et al.*, 1962) was the second atlas produced by the associates of T. Wingate Todd following his death and followed that of the hand and wrist (Greulich and Pyle, 1959). Consisting of a set of plain film radiographs, the authors determined those characteristics which were observable in a radiograph of the foot and ankle that were directly related to the process of skeletal maturation, terming these "maturity indicators" (Hoerr *et al.*, 1962). The radiographic sample was then arranged in order of apparent maturation, and 100 radiographs were selected as the most representative examples of each maturational stage. These radiographs were subsequently arranged according to the selected maturity indicators and the modal chronological age for each maturity indicator was calculated. This was carried out independently for both sexes, and was followed by collation of the radiographs and selection of those which best represented each phase of maturity. As a result, each radiographic plate included in the atlas is representative of a particular stage of maturity, with which a chronological age for females and males is associated (Hoerr *et al.*, 1962). In addition to a radiographic illustration of each maturational phase, each radiographic plate is accompanied by a description of the maturity indicators expected at each maturation phase according to discrete regions of the foot and ankle, i.e. distal tibia and fibula, hindfoot, midfoot and forefoot.

3.2.1.1 The Brush Foundation study

Initiated by T. Wingate Todd in 1928, the Brush Foundation study aimed to document the physical development of healthy children in a longitudinal study from birth to 18 years of age (Hoerr *et al.*, 1962; Nelson *et al.*, 2000). It was intended that the radiographic standards which would be produced through this study would form the basis of future teaching of juvenile skeletal development and replace those devised from studies of the skeletal remains of deceased children (Hoerr *et al.*, 1962). In addition, these radiographic standards facilitated the

diagnosis of atypical growth patterns in children by providing a basis for comparison.

The population selected for use in this study were deliberately chosen for their high socioeconomic status, high nutritional status and normal development. The initial population on which the study was based consisted of a cohort of over 4483 children from Cleveland, Ohio. This sample was enhanced by the addition of 134 children from Boston, Massachusetts, which bolstered the number of radiographs available from older individuals (Hoerr *et al.*, 1962). The Brush Foundation study cohort consisted of 50.8% females and 49.2% males. The majority of individuals included in the study were described to be of European descent, while only 7.7% were described to be of African American descent (Nelson *et al.*, 2000). Other ethnic groups represented 0.1% of the sample population.

Prior to their induction into the study, the children were the subject of medical and psychological tests, and their level of nutritional intake, medical and dental histories were recorded and maintained throughout their participation in the study. Families were asked to ensure that their child's participation in the study would continue until their 18th year and thereby provide the researchers with continuity in their sample (Hoerr *et al.*, 1962; Nelson *et al.*, 2000).

Children who were inducted into the study at birth were examined and radiographic images obtained every 3 months throughout their first year, every six months until the age of five and annually thereafter until 18 years of age. Although children were included in the study from birth, some were admitted in later childhood on the proviso they were deemed medically fit (Hoerr *et al.*, 1962). Although the data obtained through this study is invaluable, the manner in which the data was obtained would no longer be considered ethical due to the prolonged and repeated exposure of subjects to radiation. This exposure is associated with a higher health risk, particularly in young children where the patient-effective dose is higher (Teunen, 1998; Mazrani *et al.*, 2007; Frush, 2009). A longitudinal study such as this is not repeatable.

At each examination, radiographic images were obtained from multiple anatomical regions, namely the hand-wrist, the elbow, shoulder, hip, knee and ankle-foot.

These radiographs formed the bases of a series of atlases depicting the progressive skeletal maturation, including the hand/wrist, knee and foot/ankle (Greulich and Pyle, 1959; Hoerr *et al.*, 1962; Pyle and Hoerr, 1969).

3.2.2 Method for testing the radiographic atlas of skeletal development of the foot and ankle (Hoerr et al., 1962)

3.2.2.1 Main analysis method

This study was undertaken on the total study sample as outlined in section 2.2.1. It is widely acknowledged that the tempo of skeletal development differs according to biological sex (Flory, 1935; Greulich and Pyle, 1959; Hansman and Maresh, 1961; Lampl and Jeanty, 2003). Consequently, all analyses in this study were undertaken in sex-specific cohorts. As the radiographic atlas (Hoerr *et al.*, 1962) provides separate standards for females and males, it was necessary to know the sex of the individual under examination; however all assessments of age were undertaken without cognisance of the chronological age of the individual from whom the radiograph was obtained. This information was only accessed following the completion of all age assessments.

To facilitate ease of analysis, the chronological age of individuals included in the sample was converted from years to months and rounded down to the number of completed months. The difference between the assessed skeletal age based on the atlas and the chronological age was calculated by subtracting the chronological age from the assessed age. If the chronological age of the individual was greater than the estimated age, this calculation would result in a negative value, thereby indicating an underestimation of age by the radiographic atlas (Hoerr *et al.*, 1962). Conversely, if the chronological age was lower than the estimated age, the subtraction of chronological age from the estimated age would result in a positive value, indicating an overestimation of chronological age by the radiographic atlas (Hoerr *et al.*, 1962).. The mean variation between estimated age and chronological age was calculated in single year cohorts.

According to the radiographic atlas, full maturity of the foot and ankle is achieved in females by 15.2 years (Hoerr *et al.*, 1962). Following an examination of the radiographs of all individuals whose known chronological age exceeded 16 years, it was determined that skeletal maturity had indeed been achieved. Consequently,

to limit the introduction of bias in the data, 30 females aged over 16 years of age were removed from the sample. The final number of females included in the analysis was therefore 194.

All radiographs included in the original sample were examined and the skeletal age of each individual was recorded using Microsoft Excel ™ and statistical analyses conducted using Sigmaplot 12.0™.

3.2.2.2 Intra- and inter-observer analysis method

Intra-observer variation was assessed using a randomly selected subset comprising the radiographs from 30 female and 30 male left feet. Each image was reassessed in excess of 3 months after the initial assessments were undertaken and without cognisance of the chronological ages of the sample individuals or the estimated ages assigned at the first round of assessment. The subsample of images selected for the intra-observer analysis was reassessed by a second observer to determine the consistency of assessments between individuals.

The inter-observer sample included radiographs from the left feet of 30 females and 30 males. These radiographs were assessed by a second observer who was experienced in the interpretation of radiographic images and application of the radiographic atlas. The statistical significance of both the intra-observer and inter-observer analyses was calculated through the application of a one-way ANOVA.

3.2.3 Results

3.2.3.1 Intra-and inter-observer analysis

Results of the intra-observer analysis

The data resulting from the intra-observer tests were analysed through the application of a Mann-Whitney test. The results of these analyses confirmed that the variation observed in the estimated age assigned on each occasion was not statistically significant in either the female (P=0.595) or male (P=0.935) sample groups. These analyses therefore suggest that the radiographic atlas is repeatable when applied by a single assessor.

Results of the inter-observer analysis

The data resulting from the inter-observer tests were analysed through the application of a Mann-Whitney test. The results of the inter-observer analyses confirmed that the variation in the estimated age provided by each observer was not statistically significant for either the female (P=0.203; U-Statistic= 364.500) or male (P=0.847; U-Statistic=436.500) sample groups. These analyses therefore indicate that the radiographic atlas may be consistently applied by different observers.

3.2.3.2 Results of the test of the Hoerr et al. (1962) radiographic atlas in skeletal age estimation

Females

The statistical relationship between chronological age and estimated age was assessed through simple linear regression analysis. The result of this analysis is presented in Figure 3.6.

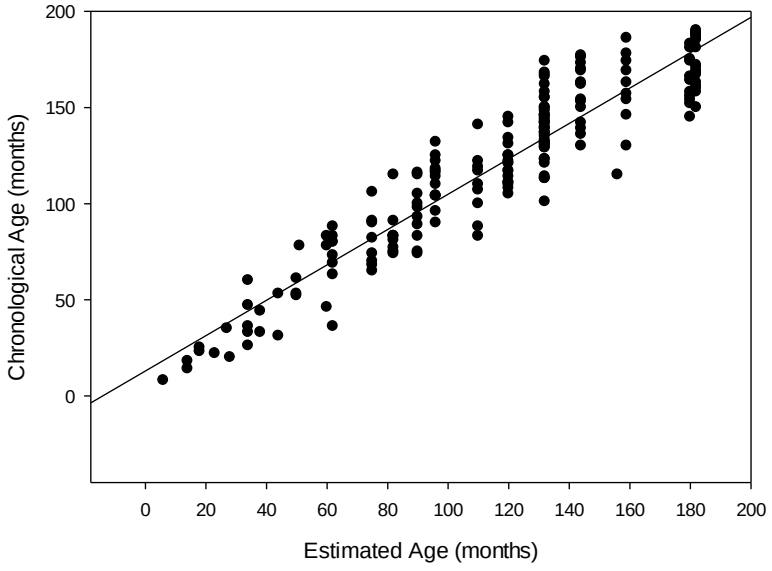

Figure 3.6: Results of the linear regression analysis of chronological age versus estimated age in female individuals (Chronological Age = 12.951+ (0.920 x Estimated Age))

When applied to data resulting from the assessment of the female sample group, a co-efficient of determination (R^2) of 0.873 was returned. This result suggests that 87.3% of variation in the chronological age is explained by variation in the estimated age. The correlation between chronological age and estimated age was determined through the application of Pearson Product Moment Correlation. This

analysis suggests that there is a statistically significant, strong positive correlation between the two factors ($r = 0.935$; $P<0.001$). The statistical significance of the variation between the estimated age and chronological age was assessed through a Mann-Whitney rank sum test. The result of this analysis showed that the two data sets were not statistically significant ($P=0.375$).

To determine the variation between chronological age and estimated age, the mean difference between the assessed skeletal ages and the chronological ages was calculated. The results of these analyses are presented in Table 3.10.

Table 3.10: Mean difference between estimated age and chronological age in female individuals within single year cohorts

Age Cohort (n=194)	Mean Over/Under Estimation of Chronological Age (months)
<1 year (n=1)	-2.00
1 year (n=5)	0.00
2 year (n=6)	2.00
3 year (n=5)	3.80
4 year (n=3)	-4.67
5 year (n=7)	-3.29
6 year (n=20)	-2.95
7 year (n=8)	-5.00
8 year (n=12)	-2.42
9 year (n=22)	-2.09
10 year (n=19)	-0.53
11 year (n=21)	-8.05
12 year (n=21)	-4.43
13 year (n=17)	0.76
14 year (n=16)	-13.00
15 year (n=11)	-6.36

The mean difference between chronological age and estimated age according to the radiographic atlas in female individuals was -3.71 months, indicating an overall under-estimation of chronological age. As the majority of values obtained from these analyses were negative, these results indicate that the chronological ages of individuals were in advance of the estimated ages according to the radiographic atlas. The mean difference between chronological age and estimated age ranged

from +3.80 months in the 3 year cohort to -13.00 months in the 14 year cohort. An over-estimation of chronological age was observed in the 2, 3 and 13 year cohorts. The greatest mean over-estimation of chronological age was observed in individuals aged 3 years, where a deviation of 3.80 months was encountered.

Males

To determine the statistical relationship between chronological age and estimated age within the male sample, a simple linear regression was conducted and the results are presented in Figure 3.7. The results of this analysis suggested that a statistically significant, strong relationship existed between chronological age and estimated age within the male sample (R^2= 0.915; P<0.001). As a result, it can be said that 91.5% of variation in the chronological age is explained by variation in the estimated age. The correlation between chronological age and estimated age was calculated through the application of Pearson Product Moment correlation. This analysis suggested that a statistically significant, strong positive relationship exists between the two factors (r=0.957; P<0.001).

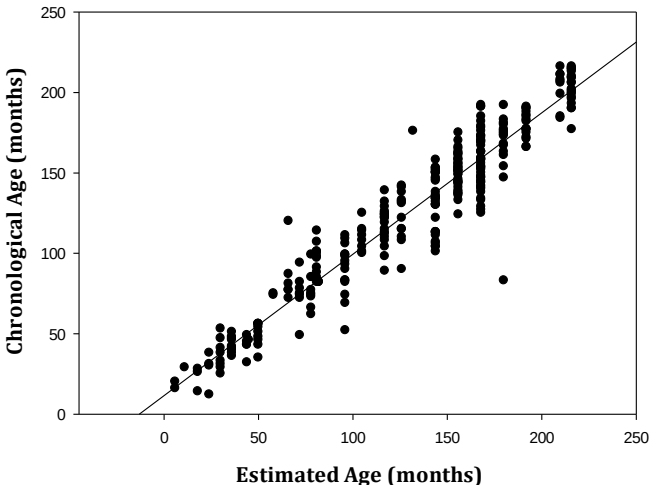

Figure 3.7: Results of the linear regression analysis of chronological age versus estimated age in male individuals (Chronological Age = 11.587 + (0.879 x Estimated Age))

Further analysis of the accuracy of the estimated age derived from the application of the radiographic atlas was undertaken through the calculation of the mean over or under estimation of the known chronological age by the estimated age. The results of these analyses are presented in Table 3.11. The mean difference between estimated age and chronological age in the male sample was +4.19

months. Further analysis showed that the mean difference between estimated age and chronological age varied between -4.50 months in the 3 year cohort and 18.33 months in the 5 year cohort. A trend of over-estimation of chronological age by the radiographic atlas was noted in all cohorts of 5 years of age and over. It was observed that the chronological ages of individuals between 1 and 4 years of age (inclusive) were under-estimated by the radiographic atlas.

Table 3.11: Mean difference between estimated age and chronological age in male individuals in single year cohorts

Age Cohort (n=194)	Mean Over/Under Estimation of Chronological Age (months)
<1 year (n=0)	--
1 year (n=4)	-2.00
2 year (n=11)	-1.82
3 year (n=18)	-4.50
4 year (n=12)	-0.75
5 year (n=3)	18.33
6 year (n=24)	3.08
7 year (n=11)	0.27
8 year (n=21)	3.95
9 year (n=21)	5.62
10 year (n=20)	4.25
11 year (n=28)	10.57
12 year (n=39)	8.36
13 year (n=27)	4.19
14 year (n=26)	2.04
15 year (n=19)	3.32
16 year (n=10)	9.90
17 year (n=14)	3.86

3.2.4 Discussion of the Radiographic Atlas of Skeletal Development of the foot and Ankle (Hoerr et al., 1962)

3.2.4.1 Discussion of intra- and inter-observer analysis

This study found that the variation between observations made by the author was not statistically different in either the female or male sample groups; however intra-observer agreement was slightly higher between male individuals than female individuals. This is consistent with the results obtained by Hackman *et al.* (2013).

Variation between observations made by multiple observers was not observed to be statistically significant in either the female or male samples. The mean difference between the estimated age and chronological age was 1.067 months for female individuals and 0.31 months for male individuals. For both intra- and inter-observer analyses, agreement between observations was found to be stronger between male samples compared with female samples, supporting the results derived from linear regression and Pearson Product Moment correlation analyses conducted on the total sample.

3.2.4.2 Discussion of the accuracy and reliability of the radiographic atlas (Hoerr et al., 1962)

It is incumbent upon any researcher undertaking an analysis of skeletal remains to ensure that the methods used are derived from an appropriate methodology and a manner of data collection which is consistent with the material on which the assessment will be carried out. It has been shown that the manner in which an assessment of age is undertaken, for example through the gross examination of dry bone or radiographic images, may influence the estimation of age as a result of the available information on which the assessment is based (Cardoso, 2008a). Consequently, application of a method derived from an alternative imaging modality or dry bone may result in an increase in the error surrounding the estimated age. For anatomical regions, such as the foot and ankle, where few radiographic methods of age assessment are available, it is critical that any and all published methods are subject to testing, with the aim of validating their applicability and accuracy, and therefore underpinning or refuting their appropriateness for use in future forensic investigations. It was therefore appropriate to subject the "*Radiographic Atlas of Skeletal Development of the Foot and Ankle*" (Hoerr *et al.*, 1962) to testing on a modern population of known chronological age. It is important to note that the "*Radiographic Atlas of Skeletal Development of the Foot and Ankle"* (Hoerr *et al.*, 1962) was developed as a means of monitoring skeletal development in a clinical context by providing standards based on healthy children who were developmentally normal. Application of this atlas in skeletal age estimation therefore amounts to applying the standards in a context for which they were not intended.

This study found there to be a strong positive relationship between the chronological age of the individuals in the sample and the estimated age according to the radiographic atlas (Hoerr *et al.*, 1962) in both female and male subjects. This relationship was found to be stronger in males than females. In both sexes, the relationship between chronological age and estimated age was found to be statistically significant. These results are consistent with those obtained by Hackman *et al.* (2013), who also found a stronger correlation between chronological age and estimated age in males than females. This finding therefore reinforces the results obtained by the inter-observer analysis undertaken by this study.

Although the strength of the correlation conveys the overall efficacy of the radiographic atlas (Hoerr *et al.*, 1962) as a method of skeletal age assessment, it is also necessary to consider the potential for variation in the estimated age relative to the chronological age of the individual. Consequently, the variation between the estimated age and chronological age was calculated by subtracting the chronological age from the estimated age. As a result, a negative value was indicative of an underestimation of chronological age, while a positive integer indicated an over-estimation of chronological age by the radiographic atlas (Hoerr *et al.*, 1962).

Analysis of the overall mean discrepancy between chronological age and estimated age in female individuals yielded a negative value, thereby indicating an under-estimation of chronological age by the radiographic atlas (Hoerr *et al.*, 1962). This overall trend supports the findings of a previous test of the radiographic atlas (Hackman *et al.*, 2013). Further analysis showed that chronological age was under-estimated by the radiographic atlas in the majority of age groups. With the exception of 3 cohorts (11, 14 and 15 years), the variation between chronological age and estimated age was found to be less than 6 months. Of those cohorts in which the variation between chronological age and estimated age exceeded 6 months, only the 14 year cohort exhibited an error in the estimated age relative to the chronological age of greater than 1 year. This corresponded to a variation of 13 months. The extent of the variation between chronological age and estimated age observed in this cohort may be explained by the structure of the radiographic

atlas (Hoerr *et al.*, 1962) in relation to the ages at which maturity levels were encountered and the subsequent radiographic plates that are available for comparison. Within the radiographic atlas (Hoerr *et al.*, 1962), the maturity observed in the foot assigned to plate 28 is recorded as occurring at approximately 13 years of age in females, while the following plate 29 is recorded at 15 years. Based on the standards presented in the radiographic atlas (Hoerr *et al.*, 1962), it is therefore not possible to assign an age of 14 years to a female individual.

In contrast to the overall trend observed in the female sample, a mean over-estimation of chronological age occurred in the 2, 3 and 13 year cohorts; however in no cohort did the variation between chronological age and estimated age exceed 6 months. Although assigned on a bone-specific basis, the standard deviations presented in the radiographic atlas corresponding to bones of the foot skeleton suggest that variation in the timing and tempo of development of at least 6 months may occur in 9 bones of the foot (Hoerr *et al.*, 1962). It is therefore considered that variation between estimated age and chronological age of less than 6 months does not exceed the range for normal variation in the development of the foot and does not constitute a prohibitive range in the context of forensic age estimation (Dreizen *et al.*, 1957).

Within the male sample, the calculation of the overall mean discrepancy between chronological age and estimated age resulted in a positive integer, thereby indicating that the radiographic atlas was likely to over-estimate the chronological age of male individuals. This finding differed from that obtained by Hackman *et al*. (2013), where an under-estimation of chronological age by the radiographic atlas (Hoerr *et al.*, 1962) was observed in both the female and male samples. Further analysis of the data suggested that within the male sample, application of the radiographic atlas to skeletal age assessment resulted in an over-estimation of chronological age in the majority of single-year cohorts.

With the exception of four cohorts (5, 11, 12 and 16 years), the mean variation between chronological age and estimated age did not exceed 6 months. As it has been considered that, in the context of an assessment of skeletal age for forensic purposes, an error of less than six months is not significant, the variation observed

between chronological age and estimated age is not considered to be prohibitive in relation to the application of the radiographic atlas in skeletal age estimation (Dreizen *et al.*, 1957; Hoerr *et al.*, 1962).

Of those cohorts where the mean variation between chronological age and estimated age exceeded 6 months, only the 5 year cohort was found to exhibit variation of greater than 1 year. This may be explained by the combined effects of the spacing between radiographic plates within the atlas and the innate variability in the ossification of the proximal phalangeal rows. When compared with the reference plates, it was observed that the ossification of some of the phalangeal epiphyses, particularly that of the proximal phalanx of the first pedal ray, was in advance of that observed in the radiographic plates. Within the radiographic atlas, the standard deviation for the ossification of this centre in the Brush Foundation study sample was recorded as 6.4 months (Hoerr *et al.*, 1962). As the only radiographic plate within the 5 year cohort represents the expected maturity of an individual aged 5.5 years, this combined with the standard deviation, may explain a large proportion of the variation between estimated age and chronological age observed in this cohort. The effect of secular change should also be considered, in that male children may be developing at a faster rate than previously observed (Hauspie *et al.*, 1997). This could also explain the pattern observed in younger cohorts where the development of skeletal maturity presented in the radiographic atlas (Hoerr *et al.*, 1962) appears to lag behind that observed in the radiographs examined during the course of this study.

In contrast to the overall trend observed within the male sample, a mean under-estimation of chronological age was observed in all cohorts between 1 year and 4 years (inclusive). Within these groups, the mean variation between chronological age and estimated age did not exceed 6 months. This study also noted that the error in age estimation increased in accordance with chronological age. This may be explained by the frequency with which radiographs were obtained from the subjects included in the construction of the radiographic atlas, where younger individuals were radiographed with greater frequency than older individuals. This facilitates the inclusion of a greater quantity of information compared with older individuals. It could also be suggested that the variability in the timing of the

pubertal growth spurt could result in a decrease in accuracy of the method in older age cohorts.

The deviation between estimated age and chronological age observed in this study may be attributable to a number of factors including those related to demographic differences between the study sample used in the development of the radiographic atlas (Hoerr *et al.*, 1962) and the sample of radiographs on which the atlas was tested, including variation in the socioeconomic status and adequate nutrition (Hackman *et al.*, 2013); and secular change in the tempo and timing of skeletal development between the period of the development of the atlas and the time at which this study was undertaken (Borkan *et al.*, 1983; Parent *et al.*, 2003; Beunen *et al.*, 2006).

The overall mean variation between chronological age and estimated age found in this study suggests that application of the radiographic atlas to skeletal age estimation may result in a more accurate estimation of age in female than male individuals. This is potentially attributable to the organisation of the original atlas as a dual standard for females and males and the advanced maturity observed in females relative to males (Hackman *et al.*, 2013). Skeletal maturity is considered, in this atlas, to have been attained in female individuals by 15.2 years of age, while an equivalent level of maturity is not observed in males until 18 years of age (Hoerr *et al.*, 1962). As a dual standard, the radiographic atlas presents an equal number of reference plates for both sexes. Consequently, the developmental progress of female individuals is more closely monitored than that of male individuals, thereby resulting in a more accurate estimation of age. This has been highlighted as an organisational flaw within the atlas (Garn and Rohmann, 1966).

The only other study known to have tested the applicability of the Hoerr *et al*. radiographic atlas (1962) is that by Hackman *et al*. (2013). This study also found a stronger correlation between estimated age and chronological age in males than females, however as this study was carried out on the same sample population as the present investigation, it may only be considered as an extended inter-observer analysis and not an independent test of the method.

3.3 Analysis of the Appearance and Fusion of the Proximal Epiphysis of the Fifth Metatarsal

As the literature related to skeletal age estimation from the foot and ankle is restricted, when presented with an opportunity to enhance the extant literature, the initiative should be seized. During the course of the analyses reported in sections 3.1 and 3.2, the presence of an epiphyseal flake was commonly noted lateral to the tuberosity of the fifth metatarsal. Although the presence of this feature has been noted in the clinical and general literature, its application in skeletal age estimation appears to be largely untested (Holland, 1921; Rogers, 1928; Abbie and Adey, 1953; Hoerr *et al.*, 1962).

As a small flake, the proximal epiphysis of the fifth metatarsal may be infrequently recovered with dry skeletal remains. This could contribute to the relative obscurity with which this centre of ossification is regarded. As the complete foot may be retained in a forensic scenario, the collection of a large number of radiographic images, of individuals of the reported age of appearance of this epiphysis, presented an ideal opportunity to assess the timing of ossification and fusion of this centre. This study will enhance the extant body of literature pertaining to skeletal age estimation from the foot and ankle.

3.3.1 Study sample

The sample of radiographs used in the assessment of the appearance and fusion of the proximal epiphysis of the fifth metatarsal comprised a subset of the complete study sample outlined in section 2.2.1. The subsample used in this analysis was based on the observations made during the completion of analyses of the Whitaker *et al.* (2002) and Hoerr *et al*. (1962) methods and comprised of radiographs from 277 individuals, including 125 females between the ages of 6 and 14 years of age and 152 males between 8 and 15 years of age. In addition to the exclusionary criteria detailed in section 2.2.1, individuals who exhibited a fracture across the region of the epiphysis on the proximal end of the fifth metatarsal were omitted from this section of the study. The distribution of the sample according to sex and chronological age is presented in Figure 3.8.

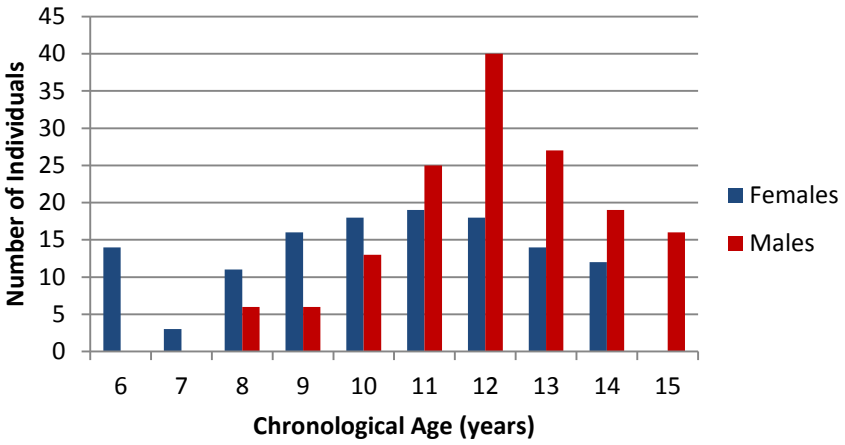

Figure 3.8: Distribution of the sample for the study of the proximal epiphysis of the fifth metatarsal according to biological sex and chronological age

3.3.2 Methods

3.3.2.1 Method for the assessment of the appearance and fusion of the proximal epiphysis of the fifth metatarsal

Each radiograph was re-examined and the presence and state of fusion of this epiphysis was scored according to the criteria presented in Table 3.12. Graphical illustrations of each maturity stage are presented in Figure 3.9.

Table 3.12: Criteria for scoring the ossification and fusion of the proximal epiphysis of the fifth metatarsal

Maturity Stage	Criteria
0	Ossification centre absent.
1	Ossification centre present but fusion has not commenced.
2	Fusion is on-going.
3	Fusion is complete and fusion line obliterated

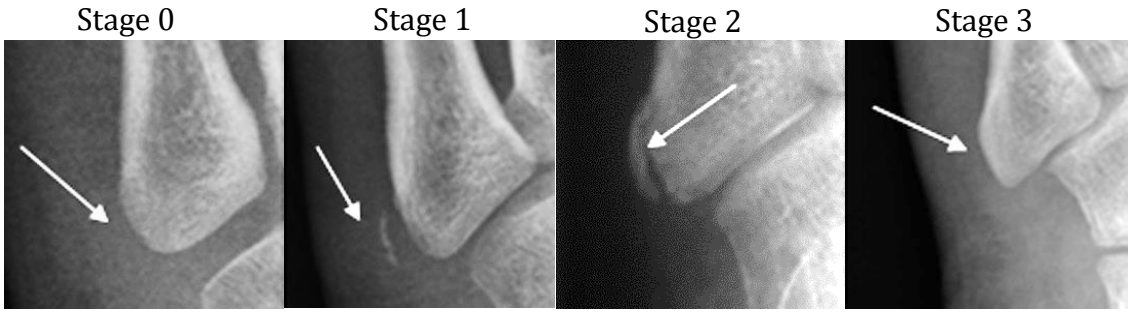

Figure 3.9: Maturity stages of the proximal epiphysis of the fifth metatarsal

Following the examination of all radiographs, the percentage of individuals within each single-year cohort to whom each maturity stage was assigned was calculated. In addition, the maximum and minimum ages of individuals assigned each maturity stage was recorded. The mean age of appearance and fusion was then calculated for males and females within this study sample and compared with the values in published literature (Hoerr *et al.*, 1962; Scheuer and Black, 2000).

3.3.2.2 Method for the intra-observer and inter-observer analysis

A subset of radiographic images was established from which intra-observer reliability would be determined. As, in the main analysis, no epiphysis had appeared in females younger than 8 years of age, the individuals included in the intra-observer analysis ranged between 8 and 14 years of age. Similarly, as no epiphyses were observed in males younger than 10 years of age, the male individuals included in the intra-observer study ranged between 10 and 15 years of age. Images included in this sample were selected randomly and represented 6 individuals from each year cohort, resulting in a total sample of 78 individuals including 36 males and 42 females.

A second set of images was selected for analysis to determine the inter-observer reliability. This subset consisted of radiographs from 50 individual (25 males and 25 females). All assessments were carried out without cognisance of the chronological age of the individual or the score assigned on a previous occasion or by an alternative individual.

3.3.3 Results

3.3.3.1 Intra- and inter-observer analysis of the appearance and fusion of the proximal epiphysis of the 5th metatarsal

Results of the intra-observer analysis

Analysis of the intra-observer data was undertaken to determine the degree of agreement between the first and second assessments carried out by the first author. The variation in the maturity stage assigned in the first and second rounds of assessment carried out on the male and female samples are presented in Figure 3.10.

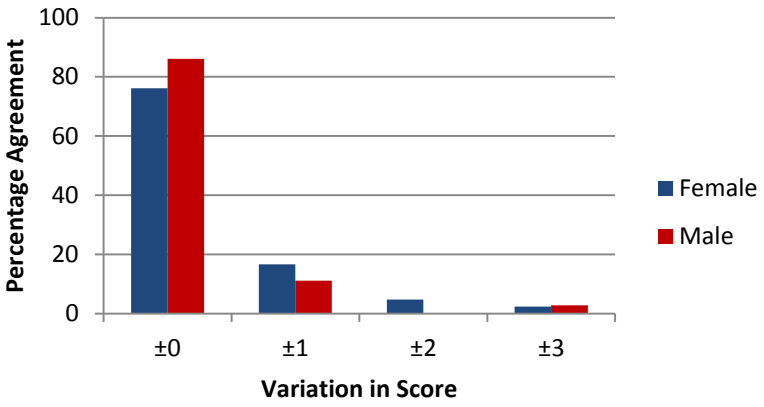

Figure 3.10: Percentage intra-observer variation in maturity stage assigned to the proximal epiphysis of the fifth metatarsal according to biological sex

These assessments suggest that repeat observations carried out by the same individual yielded equivalent maturity stages on 76.2% of occasions for females and 86.1% of occasions for males. The scores assigned during the first and second rounds of assessment varied by a single maturity stage in 16.7% of females and 11.1% for males.

The statistical significance of the variation between observations was calculated through the application of Mann-Whitney Rank Sum analyses. The results of these analyses are presented in Table 3.13.

Table 3.13: Results of Mann-Whitney Rank Sum test for intra-observer analysis

Sex	n.	P-Value	T-Value	U-Statistic
Female	42	0.626	1734.000	831.000
Male	36	0.491	1373.000	589.000

These data showed that there was no statistically significant difference between the maturity scores assigned on the first and second rounds of assessment. This indicates that the scoring system for assessing the degree of ossification and fusion of the proximal epiphysis of the fifth metatarsal is repeatable when applied by a single observer.

Results of the inter-observer analysis

Analysis of the data obtained from the assessments carried out by the first and second authors was undertaken to test the consistency of the method. The

percentage agreements between the scores assigned by two observers for the male and female samples are presented in Figure 3.11.

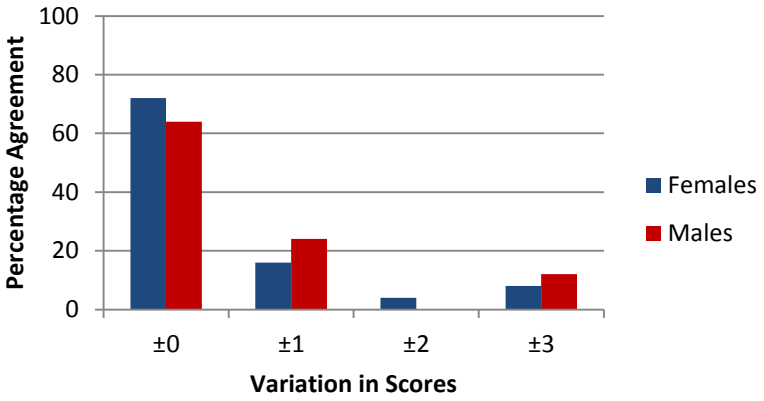

Figure 3.11: Percentage inter-observer variation in maturity stage assigned to the proximal epiphysis of the fifth metatarsal according to biological sex

These results suggest that multiple observers are likely to agree on 72% of occasions for females and 64% of occasions for males. The maturity scores assigned by two observers differed by a single maturity score on 16% of occasions in females and 24% of occasions in males. It was also observed that the assigned maturity stages varied by 3 stages in 8 % and 12% of occasions for females and males respectively.

A Mann-Whitney Rank Sum analysis was also undertaken to determine the statistical variation between the scores assigned by the first and second observers within the female and male groups. The results of these analyses are presented in Table 3.14.

Table 3.14: Results of Mann-Whitney Rank Sum test for inter-observer analysis

Sex	n.	P-Value	T-Value	U-Statistic
Female	25	0.960	634.500	309.500
Male	25	0.785	623.500	289.500

These results show that the variation between maturity scores assigned by different observers was not statistically significant in either sex cohort. It may therefore be suggested that the criteria presented for the assessment of appearance and fusion of the proximal epiphysis of the fifth metatarsal are repeatable when applied by different observers.

Overall, intra-observer agreement was found to be marginally greater than inter-observer consistency in both sex cohorts; however further analysis suggested that the variation between intra-observer and inter-observer assessments was not statistically significant in either female (P=0.212) or male (P=0.052) samples.

3.3.3.2 Results of the analysis of the appearance and fusion of the proximal epiphysis of the fifth metatarsal

Females

The results of the analysis of ossification and fusion of the proximal epiphysis of the fifth metatarsal in female individuals are presented in Table 3.15.

Table 3.15: Percentage of female individuals represented by each stage of ossification of the proximal epiphysis of the fifth metatarsal according to chronological age

	Maturity Stage				Total percentage of cohort exhibiting epiphysis
Chronological Age (years)	**0**	**1**	**2**	**3**	
6 (n=14)	100	0	0	0	0
7 (n=3)	100	0	0	0	0
8 (n=11)	81.8	0	18.2	0	18.2
9 (n=16)	62.5	37.5	0	0	37.5
10 (n=18)	44.4	11.1	33.3	11.1	55.5
11 (n=19)	0	36.8	31.6	31.6	100
12 (n=18)	0	16.7	22.2	61.1	100
13 (n=14)	0	7.1	0	92.9	100
14 (n=12)	0	0	0	100	100

The results of this study suggest that in females, the appearance and fusion of this ossification centre occurs in a relatively predictable pattern between the ages of 8 and 14 years. The presence of an ossified flake epiphysis of the proximal fifth metatarsal was first observed in female individuals aged 8 years. All individuals of 11 years of age and over exhibited an ossified epiphyseal flake at various stages of fusion. Active fusion occurred between 8 and 12 years of age. Complete fusion of the epiphysis was first observed in females of 10 years of age and was completed in all subjects by 14 years of age.

Males

The results of the analysis of the ossification and fusion of the proximal epiphysis of the fifth metatarsal in male individuals are presented in Table 3.16.

Table 3.16: Percentage of male individuals represented by each stage of ossification of the proximal epiphysis of the fifth metatarsal according to chronological age

	Maturity Stage				Total percentage of cohort exhibiting epiphysis
Chronological Age (years)	**0**	**1**	**2**	**3**	
8 (n=6)	100	0	0	0	0
9 (n=6)	100	0	0	0	0
10 (n=13)	67	23	0	0	23.08
11 (n=25)	52	28	12	8	48
12 (n=40)	20	27.5	45	7.5	80
13 (n=27)	18.5	14.8	40.7	25.9	81.48
14 (n=19)	5.3	5.3	26.3	63.2	94.74
15 (n=16)	0	0	0	100	100

The centre of ossification for the proximal epiphysis of the fifth metatarsal was first observed in males aged 10 years and was observed in some individuals as an unfused ossified flake until 14 years of age. . Active fusion was observed to occur in individuals between 11 and 14 years of age. Completion of fusion was first observed in males aged 11 years however fusion was not completed in all individuals within a cohort until 15 years of age.

Individuals of unknown sex

If the sex of the individual is unknown, as may often be the case in forensic investigations, the appearance and fusion times for females and males must be combined to provide an estimated age range suitable for application, irrespective of sex. From this study, it is suggested that when sex is unknown, a stage of 0 may be interpreted as an indication of a chronological age of 14 years or younger; stage 1 is indicative of an individual between 9 and 14 years, stage 2 is indicative of an individual between 8 and 14 years; and stage 3 indicates a chronological age of 10 years or older. These results are summarised in Table 3.17.

Table 3.17: Age ranges in years associated with maturity stages for females, males and individuals of unknown sex.

	Maturity Stage			
Sex	**0**	**1**	**2**	**3**
Female	≤10	9-13	8-12	≥10
Male	≤14	10-14	11-14	≥11
Unknown	≤14	9-14	8-14	≥10

3.3.4 Discussion of the proximal epiphysis of the fifth metatarsal as a tool in skeletal age estimation

3.3.4.1 Discussion of intra- and inter-observer analysis

The methodological approach used in this study was tested through the analysis of the intra- and inter-observer reliability. These analyses suggested that intra-observer reliability was likely to be stronger than inter-observer reliability within the male sample. Analysis of the results of intra- and inter-observer reliability within the female sample however suggested that inter-observer agreement was marginally greater than intra-observer agreement. In those cases where variation between assessed stages was observed, the majority of variation was by a single stage. This is most likely to have occurred between stage 2 and stage 3, thereby reflecting indecision on the part of the observer as to when to consider epiphyseal fusion to be complete.

Within the analysis of inter-observer variation, it was found that a small minority of observations were discordant by three scores and can therefore only be explained by variation in the assignment of scores 0 and 3. This is perhaps attributable to inexperience in the interpretation of the radiographic appearance of the metatarsal prior to the appearance of the epiphysis in comparison with the outline of the mature bone. As the results of the intra-observer analysis showed fewer incidents where variation between observation disagreed by greater than 1 maturity score than was encountered in the inter-observer analysis, it is suggested that greater experience in application of the method may increase awareness of the immature and mature radiographic outline of the proximal end of the fifth metatarsal.

3.3.4.2 Discussion of the chronology of ossification and fusion of the proximal epiphysis of the fifth metatarsal

The foot and ankle, unlike the hand and wrist, has largely been overlooked by the age estimation literature over the past half century. Consequently, it is necessary to ensure that all information that is pertinent to the estimation of age is collated and employed in the assessment of chronological age when presented with a limited quantity of remains.

To investigate the potential utility of the proximal epiphysis of the fifth metatarsal in juvenile skeletal age estimation, a radiographic study of the timing of appearance and fusion of this epiphysis was undertaken using a numerical scoring method. This approach has been widely used in the assessment of age from both dry bone (Schaefer and Black, 2005) and radiographic methods (Schulz *et al.*, 2005; O'Connor *et al.*, 2008). For this study, four stages were considered optimal as fewer stages would result in a decrease in precision while a greater number of stages would introduce ambiguity into the assessments and therefore would be likely to increase intra- and inter-observer error (MacLaughlin, 1987; Whitaker *et al.*, 2002).

Through the examination of radiographs from 277 individuals, this study observed that the ossification and fusion of the proximal epiphysis of the fifth metatarsal followed a relatively predictable pattern in both females and males. The epiphysis was observed to commence ossification between the ages of 7 and 11 years in females and 10 and 15 years in males. Although the age at which ossification of the epiphysis was first observed differed between females and males, the duration of activity was similar in both sex groups. The variation in timing of appearance between females and males is attributable to the accepted temporal divergence between the sexes in terms of osteological development and is in agreement with existing literature which reports female skeletal development to be in advance of males by approximately 2 years (Flory, 1935; Fishman, 1982; Cardoso, 2008a).

The cross-sectional nature of the study limits the interpretations that may be made from the progressive fusion of the epiphysis. The pattern observed in the percentage of individuals at each maturity stage however suggests that the epiphysis is likely to be undergoing active fusion in females between 8 and 13

years of age and in males between 11 and 14 years of age and will be complete in females by 14 years of age and males by 15 years of age.

Prior to the development of secondary sexual characteristics, determination of sex from skeletal remains is considered to be unreliable (Wilson *et al.*, 2008). Consequently, in the event that remains are recovered and require an estimation of chronological age, without the use of DNA testing, the sex of the individual may be unknown at the time the age assessment was carried out. It is therefore necessary to present the results of this study in a form which may be applied irrespective of the biological sex of the individual. Due to the precocity of female skeletal development relative to males (Cardoso, 2008a), it is necessary to provide composite age ranges which incorporate the age ranges assigned to both sex groups. It should be noted however that the timing of ossification and fusion exhibit a degree of variation and so the results of this study may be better applied in their most basic form. This summary would suggest that if no ossified epiphysis is located, regardless of the stage of fusion, the individual is likely to be aged 10 years or younger. The presence of an epiphysis, in any stage of fusion indicates a child who is 10 years or older.

The timings of ossification and fusion of the proximal epiphysis of the fifth metatarsal observed during this study appear to support those published by Hoerr *et al*. (1962) in the "*Radiographic Atlas of Skeletal Development of the Foot and Ankle*" which suggest that the epiphysis is likely to appear in females at 9.7 years ±1.2 years and fuse at 11.7 years ±1 year and in males at 12.1 years ±1.3 years with fusion occurring at 14.2 years ±1.1 year. These values suggest an appearance range of 8.5 years-11.9 years and a fusion range of 10.7 years to 12.7 years in females. The estimated timings of appearance and fusion presented by Hoerr et al. encompass those published by Scheuer and Black (2000), where it is suggested that the epiphysis may appear between 9 and 10 years in females and between 12 and 13 years in males, with fusion occurring over the following 2 years. It is acknowledged that this centre of ossification is unlikely to be identifiable in isolation, and so it is presumed that these timings are based on a radiographic reference, although this is not provided in the text. These data support the

findings of this study in relation to the overall timing of appearance and fusion of the proximal epiphysis of the fifth metatarsal.

In accordance with the general principles of relative retardation of skeletal growth in males compared with females, the appearance range for males spans between 10.8 years and 13.4 years in males with fusion occurring between 13.1 years and 15.3 years (Flory, 1935; Cardoso, 2008a). These values are consistent with those obtained from the analysis of appearance and fusion times observed in this study population sample.

3.4 Conclusion

In terms of the distribution of published methods for estimating chronological age, there is a paucity of methods based on the foot and ankle when compared with other anatomical regions. Much of the literature relating to juvenile age assessment from the foot is derived on the analysis of dry bone samples rather than radiographic studies (McKern and Stewart, 1957). It has been shown that the timing of epiphyseal fusion as perceived from the dry bone differs from that observed during radiographic assessment (Cardoso, 2008a). Consequently the standards derived from one methodology or imaging modality should only be applied to images consistent with those on which they were based (Schulz *et al.*, 2008a). As so few methods of radiographic assessment of age from this region exist, it is essential that repeated testing and validation of radiographic methods are undertaken (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002). This will ensure that only those methods which fulfil the requirements of accuracy, reliability and repeatability, necessary for the production of scientific evidence with sufficient probative value as suggested by The Law Commission of England and Wales (2011), are recommended for use in forensic investigations. It is also the responsibility of researchers and practitioners to consider all potential sources of information relating to the estimation of chronological age and develop novel approaches to the assessment of skeletal age which may serve to augment existing methods.

When presented with the challenge of estimating chronological age from the skeleton, it is necessary to consider which of the available methods is the most

appropriate for use. This appears to be the first study which sought to establish the validities of the Whitaker *et al*. (2002) and the Hoerr *et al.* (1962) methods of age estimation. As the only methods currently available for estimating age from this anatomical region, it was imperative that their validity be tested and only those methods which are fit for purpose are implemented in forensic investigations.

Study Phase 2 – The Persistence of Epiphyseal Scars in Adult Individuals

The following chapters of this thesis represent the findings of the body of work conducted between September 2011 and July 2013 under Ordinance 39 of the Charter of the University of Dundee.

4 Persistence of the Epiphyseal Scar in the Proximal Humerus

4.1 Sample distribution

The distribution of the proximal humerus study sample is presented in Table 4.1 according to chronological age, biological sex and side of the body.

Table 4.1: Distribution of the sample used in the analysis of the proximal humerus according to chronological age, biological sex and side of the body

Age	Female Right	Female Left	Male Right	Male Left
20	5	5	5	5
21	5	5	5	5
22	5	5	5	5
23	5	5	5	5
24	5	5	5	5
25	5	5	5	5
26	5	5	5	5
27	5	5	4	5
28	5	5	5	5
29	5	5	5	5
30	5	5	5	5
31	5	5	5	5
32	5	5	5	5
33	5	5	5	5
34	5	5	5	5
35	5	5	5	5
36	5	5	5	5
37	5	5	5	5
38	5	5	5	5
39	5	5	5	5
40	5	5	5	5
41	5	5	5	5
42	5	5	5	5
43	5	5	5	5
44	5	5	5	5
45	5	5	5	5
46	5	5	5	5
47	5	5	5	5
48	5	5	5	5
49	5	5	5	5
50	5	5	5	5
Total	**155**	**155**	**154**	**155**

4.2 Results

4.2.1 Intra-observer analysis

Initially, a series of ANOVA were undertaken to assess the variation in the assignment of TPS by a single observer on multiple occasions. These analyses

suggested that there was no statistically significant variation in the TPS assigned to individuals within the female (P=0.891) or male (P=0.542) samples.

The distributions of the variation between observations made by a single observer are presented in Figure 4.1. Analysis of the data from the intra-observer assessments suggested that 86.67% of scores assigned to females and 80% of scores assigned to males were within two scores of those assigned during the first round of assessment.

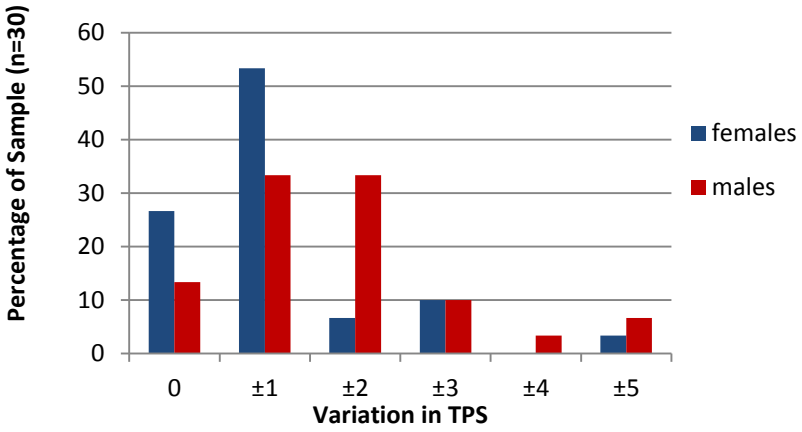

Figure 4.1: Intra-observer variation in Total Persistence Score assigned to the proximal humerus according to biological sex

To assess the statistical relationship between the TPS assigned during the first and second rounds of assessment, a GLM analysis was undertaken, the results of which are presented in Table 4.2.

Table 4.2: Results of the General Linear Model analysis of intra-observer variation in the proximal humerus

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	<0.001	0.322	0.104	0.096	9.6%
Round	0.859	0.000	0.000	-0.008	0%
Sex*Round	0.495	0.327	0.107	0.084	8.4%

This analysis showed that there was no significant difference between the TPS scores assigned during the first and second rounds of assessment when considered as either a single factor (P=0.859) or as a co-varying factor when considered with biological sex (P=0.495). The results of these analyses suggest that any variation that exists between TPS assigned by the same observer on two occasions is not

statistically significant and therefore suggest that this method is consistent when applied by a single observer.

4.2.2 Inter-Observer Analysis

Initially, a one-way ANOVA was undertaken to determine the significance of any variation that existed between the TPS values assigned to sex specific cohorts. The results of this analysis indicated that the variation between the TPS values assigned to females and males by three observers was statistically significant P=0.047; H=3.963). Following this result, all analyses were undertaken in sex-specific groups. An additional series of one-way ANOVA were conducted to assess the overall statistical significance of the variation between the TPS values assigned by three observers within sex-specific cohorts. The results of these analyses showed that while the variation within the TPS values assigned to female individuals was not statistically significant (P=0.713); a statistically significant degree of variation was observed within the male sample (P=0.012).

In addition to establishing the overall level of significance of the variation between TPS values assigned by different observers, it was prudent to establish the percentage agreement between the observers. These data are presented in Table 4.3.

Table 4.3: Inter-observer percentage agreement in Total Persistence Score in the proximal humerus

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	80.00	80.00	93.33
Male	83.33	83.33	86.67

The greatest percentage agreement was found between the TPS values assigned by observers 2 and 3 in both the female and male samples; however the percentage agreement between assessments in the female sample was found to be greater than that of the male sample. As these observers represented the highest levels of experience, these findings suggest that experience in the interpretation of radiographic images may be beneficial to the inter-observer reliability of the scoring system. The percentage agreements between observers 1 and 2 and 1 and 3 were found to be equivalent in both the female and male samples. The statistical

significance of the variation between the TPS values assigned by multiple observers was calculated through the application of a series of one-way ANOVA. The results of these analyses are presented in Table 4.4.

Table 4.4: Statistical significance of the inter-observer variation in the assignment of Total Persistence Scores in the proximal humerus according to biological sex

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	0.472	0.390*	0.596
Male	0.006*	0.272*	0.048*

*statistical power α <0.8

These results show that within the female sample, no statistically significant difference was found between the TPS values assigned by multiple observers. This finding was not replicated in the male sample, where statistically significant differences were found between the TPS value assigned by observers 1 and 2; and 2 and 3.

To further investigate the statistical relationship between observer and TPS, a series of GLM analyses were undertaken. The results of these analyses are presented in Table 4.5.

Table 4.5: Results of the General Linear Model analysis of inter-observer variation in the proximal humerus

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	0.057	0.14	0.020	0.015	1.5
Observer	0.017	0.21	0.045	0.034	3.4
Sex*Observer	0.305	0.28	0.078	0.051	5.1

The results of the GLM analysis suggest that inter-observer variation exerts a statistically significant influence on the assignment of TPS (P=0.017). Although this relationship was statistically significant, variation in observer explained only 3.4% of variation in TPS. An assessment of the combined influence of sex and observer on TPS found that although this model explained the greatest degree of variation in TPS (R^2=0.0051), the relationship was not statistically significant (P=0.305). These results suggest that application of the scoring system results in statistically repeatable assignment of TPS.

4.2.3 Main data analysis

A Shapiro-Wilk test was conducted to determine the statistical normality of the distribution of TPS data derived from the assessment of the proximal humerus. The result of this analysis showed that neither the female (P= <0.001; W-statistic= 0.961) nor the male (P=<0.001; W-statistic = 0.955) data sets were normal in their distribution.

The TPR for the female and male samples were calculated. Overall, 94.19% of females and 94.82% of males were observed to exhibit some remnant of an epiphyseal scar in the proximal humerus. The distributions of the female and male samples are presented according to sex and TPS in Figure 4.2. Although the potential maximum TPS was 12, only a single female individual was observed to retain an epiphyseal scar of TPS ≥9; no male was assigned a TPS value greater than 8. Consequently, the x-axis of Figure 4.2 has been limited to reflect the maximum assigned TPS value.

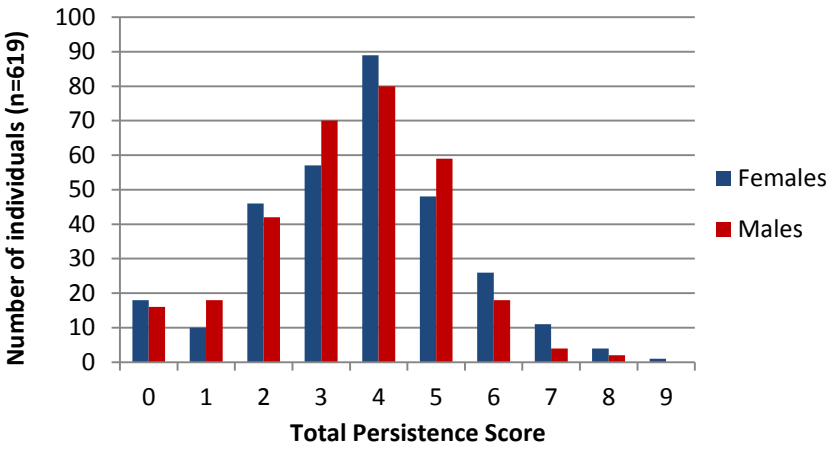

Figure 4.2: Distribution of the proximal humerus study sample according to biological sex and Total Persistence Score

To further examine the distribution of the data, the mean, maximum and minimum chronological ages of the individuals for whom each TPS value was assigned were calculated and the results presented in Table 4.6 and Table 4.7 for females and males respectively.

Table 4.6: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the proximal humerus in female individuals

Total Persistence Score	Mean (years)	Maximum (years)	Minimum (years)
0	36.50 (n=18)	50	23
1	34.70 (n=10)	43	22
2	35.28 (n=46)	50	20
3	36.53 (n=57)	50	21
4	36.20 (n=89)	50	20
5	34.54 (n=48)	50	20
6	30.50 (n=26)	48	20
7	31.36 (n=11)	45	22
8	25.75 (n=4)	35	20
9	20.00 (n=1)	20	20
10	--		
11	--		
12	--		

Table 4.7: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the proximal humerus in male individuals

Total Persistence Score	Mean (years)	Maximum (years)	Minimum (years)
0	31.50 (n=16)	50	25
1	36.67 (n=18)	50	25
2	35.38 (n=42)	50	22
3	33.89 (n=70)	50	20
4	35.63 (n=80)	50	20
5	35.03 (n=59)	50	20
6	30.61 (n=18)	49	20
7	31.75 (n=4)	38	20
8	37.50 (n=2)	42	33
9	--		
10	--		
11	--		
12	--		

The results of this analysis suggest that individuals are more likely to be assigned a TPS value <6 than they are to be assigned a TPS value of ≥6, irrespective of biological sex. As the TPS value assigned represents a scale against which trends in the mean chronological age of individuals within a cohort may be assessed, the net difference in chronological age between cohorts 1 and 6 was calculated in both sexes. These cohorts were selected as they represent the highest TPS values common to both sexes where n>10. As the net difference between the mean chronological ages in male individuals (-6.06 years) and female individuals (-4.20

years) were negative, the results of these analyses suggest that an inverse relationship may exist between mean chronological age and TPS as in both sexes.

To assess the relationship between obliteration of the epiphyseal scar and chronological age, the percentage of individuals within each cohort to whom a TPS value of 0 was assigned was calculated for the female and male samples. Following this analysis, linear regression analyses were conducted to assess the strength of the relationship between these factors. The results of these analyses are presented in Figure 4.3 and Figure 4.4 for females and males respectively.

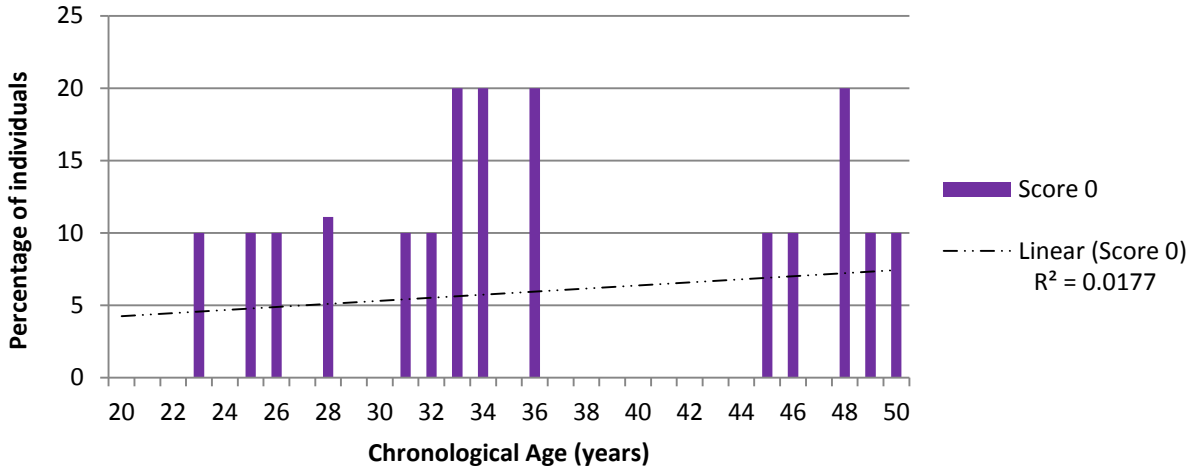

Figure 4.3: Percentage of female individuals exhibiting complete obliteration of the epiphyseal scar in the proximal humerus according to chronological age

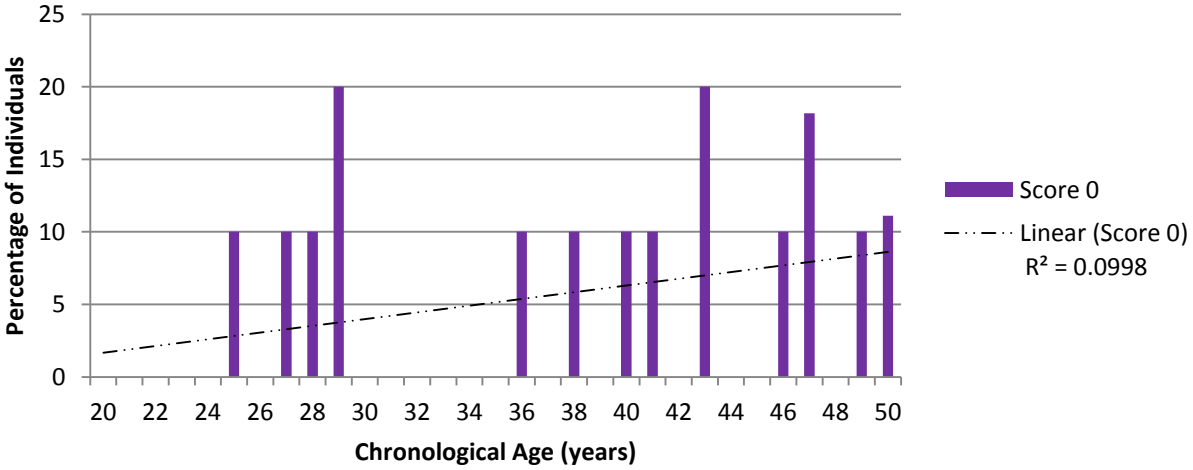

Figure 4.4: Percentage of male individuals exhibiting complete obliteration of the epiphyseal scar in the proximal humerus according to chronological age

The results of these analyses suggest that in both females and males, the frequency of complete obliteration is between 10% and 20% across the extent of the chronological ages included in this sample. Within this analysis, the application of linear regression analyses facilitated the assessment of the statistical relationship between increasing chronological age and complete obliteration of the epiphyseal scar. These analyses showed that a positive relationship exists between these factors in both the female and male samples, with the strength of the interaction was stronger in males (R^2=0.0998) than in females (R^2=0.0177) but both were relatively weak.

To assess the statistical relationship between the level of persistence of the epiphyseal scar and the biological factors of age, sex and side of the body, a series of GLM analyses were conducted, the results of which are presented in Table 4.8.

Table 4.8: Results of the General Linear Model analyses in the proximal humerus

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.038	0.072	0.025	2.50
Sex	0.144	0.003	0.002	0.20
Side	0.765	0.000	-0.001	-0.10
Age*sex	0.151	0.135	0.040	4.00
Age*side	0.693	0.113	0.016	1.60
Sex*side	0.477	0.044	0.000	0.00
Age*sex*side	0.365	0.228	0.036	3.60

The results of the GLM analysis suggest that when considered as an independent variable, a statistically significant relationship was found between chronological age and TPS (P=0.038); however the strength of this interaction is low (R^2=0.025). As neither sex (P=0.144) nor side of the body (P=0.765) were observed to exhibit a significant relationship with TPS, it is suggested that these factors do not influence the persistence of the epiphyseal scar in the proximal humerus to a statistically significant degree. Further GLM analyses showed that no subsequent interactions between multiple factors exhibited statistically significant relationships with TPS.

To assess the variation in the persistence of the epiphyseal scar across the proximal humerus, a series of analyses were undertaken to calculate the distribution of the sample according to RPS values within the medial (tracks 1 and 2), central (tracks 3 and 4) and lateral (tracks 5 and 6) thirds of the bone. Initially,

the mean RPS value for each region was calculated for both sex cohorts. These data, presented in Table 4.9, showed that in both sex cohorts, the highest mean RPS value occurred in the central region while the lowest mean RPS value was observed in the medial region.

Table 4.9: Mean regional persistence scores for females and males in the proximal humerus

	Medial Region	Central Region	Lateral Region
Female	0.75	1.74	1.16
Male	0.76	1.84	0.89

With the exception of the lateral region, the mean persistence of the epiphyseal scar was greater in male individuals than female individuals. To further investigate the distribution of RPS values within the study sample, the percentage of individuals to whom each RPS value was assigned in each region of the proximal humerus was calculated for females and males. These data are presented in Table 4.10 and Table 4.11 for females and males respectively.

Table 4.10: Percentage distribution of Regional Persistence Scores in the proximal humerus in female individuals.

Persistence Score	Medial Region	Central Region	Lateral Region
0	39.35	10.32	21.29
1	48.06	18.06	44.84
2	10.65	60.65	30.32
3	1.94	9.35	3.55
4	0.00	1.61	0.00

Table 4.11: Percentage distribution of Regional Persistence Scores in the proximal humerus in male individuals

Persistence Score	Medial Region	Central Region	Lateral Region
0	35.28	8.09	32.36
1	55.02	15.86	46.60
2	8.09	60.19	20.39
3	1.62	15.86	0.65
4	0.00	0.00	0.00

The greatest percentages of individuals to whom persistence scores of 0 or 1 were assigned were found in the medial region for both females and males. Within the medial region, 87.41% and 90.3% of females and males respectively were assigned a persistence score of ≥1. The greatest percentages of individuals to whom

persistence scores of 2 or 3 were assigned were found in the central region where 70% of females and 76.05% of males were assigned a TPS value of 2 or 3. A persistence score of 4 was only observed in the central region of the female sample, where 1.61% of individuals were found to exhibit a complete epiphyseal scar in the central third of the proximal humerus.

To assess the statistical significance of the variation between the RPS values assigned to females and males in the proximal humerus, a series of one-way ANOVA were conducted. These analyses suggested that there was a statistically significant difference between females and males in the lateral third of the proximal humerus ($P<0.001$; $H=17.192$). Within the medial ($P=0.660$; $H=0.194$) and central ($P=0.071$; $H=3.265$) thirds of the bone, no statistically significant variation in the assignment of persistence scores between females and males was found.

A series of one-way ANOVA were conducted to assess the statistical significance of the variation in persistence score between the medial, central and lateral thirds of the proximal humerus. The results of these analyses suggested that statistically significant variation was present between all regions within the female and male data sets. Within the female sample, each interaction was statistically significant ($P<0.001$). Within the male sample, the variation between the medial and central areas and the central and lateral areas were significant ($P<0.001$). The interaction between medial and lateral thirds of the bone was statistically significant ($P=0.022$). This result suggests that there is a greater degree of similarity between the medial and lateral aspects of the male proximal humerus than between the central third of the bone and either the medial or lateral thirds.

To further examine the relationship between persistence of the epiphyseal scar within the discrete regions of the proximal humerus and the biological parameters included in this study, a GLM analysis was conducted, the results of which are presented in Table 4.12. These analyses suggest that when considered independently, both chronological age ($P=0.001$) and region of the bone ($P<0.001$) exhibited a statistically significant relationship with persistence of the epiphyseal scar; however the coefficient of determination of the relationship between region

and persistence of the epiphyseal scar (0.247) greatly exceeded that derived from the analysis of the interaction between chronological age and persistence of the epiphyseal scar (0.016). Further analyses yielded only a single statistically significant relationship. This was found between region of the bone and biological sex ($P<0.001$; $R^2=0.255$). As this interaction explained a greater percentage of variation in persistence of the epiphyseal scar than region when considered independently, the interaction between region of the proximal humerus and biological sex represents the best explanatory model for the regional variation in the persistence of the epiphyseal scar in this anatomical area.

Table 4.12: Results of the General Linear Model analyses for regional variation in persistence of the epiphyseal scar in the proximal humerus

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.001	0.032	0.016	1.6
Sex	0.191	0.001	0.000	0
Side	0.970	0.000	-0.001	-0.01
Region	<0.001	0.248	0.247	24.7
Age*sex	0.059	0.056	0.024	2.4
Age*side	0.629	0.046	0.014	1.4
Sex*side	0.625	0.001	-0.001	-0.01
Region*side	0.048	0.251	0.249	24.9
Region*sex	<0.001	0.257	0.255	25.5
Region*age	0.758	0.301	0.265	26.5
Age*sex*side	0.071	0.093	0.028	2.8
Region*side*sex	0.305	0.261	0.256	25.6
Region*side*age	0.629	0.333	0.259	25.9
Region*sex*age	0.919	0.350	0.278	27.8
Region*sex*age*side	0.973	0.421	0.277	27.7

4.3 Discussion of the persistence of the epiphyseal scar in the proximal humerus

4.3.1 Discussion of intra-observer and inter-observer analysis in the application of the method to the proximal humerus

As this study represents the first attempt to examine the persistence of the epiphyseal scar in the proximal humerus within an adult population, it was necessary for an assessment of the intra-observer and inter-observer consistency to be undertaken in the application of the scoring system presented in this study to this anatomical region.

This study found that the variation between TPS values assigned by a single observer on two occasions was not statistically significant in either female or male individuals; however as the statistical power of the analysis of intra-observer variation within the male sample did not reach the threshold for sufficient statistical power ($\alpha=\leq 0.8$), there is an increased risk of a Type II error. Consequently, although these findings indicate that the scoring system for assessing the persistence of epiphyseal scars presented in this study is consistent when applied by a single observer on different occasions, they cannot be considered definitive.

Within the female sample, 86.67% of TPS values assigned during the second round of assessments were within 2 scores of those assigned at the first round of assessment. In the male sample, the percentage intra-observer agreement decreased to 80%. As all assessments of radiographs from the male sample were completed after that of the female sample, these results indicate that experience in the application of the method may not influence the degree of intra-observer consistency.

It is not only imperative that the scoring system introduced by this study is repeatable when applied by a single individual but that, when used by multiple practitioners the repeatable nature of the assessment is maintained. The results of this study indicated that although the TPS values assigned by the three observers did not differ significantly in the female sample, a statistically significant degree of variation between the TPS values assigned by three observers was encountered in the male sample. Although these data suggest the overall trend observed in the inter-observer analysis, it was necessary to establish the variation between individual pairs of observers. As the observers employed in this study represented varying levels of experience in radiographic interpretation and skeletal age estimation, analysis of the variation between pairs of observers also facilitated an examination of the effect of experience on the application of the method. From these analyses, no statistically significant variation was observed in the assignment of TPS in the female sample. While this pattern was maintained for observers 1 and 3 in the male sample, the variation in the TPS values assigned by observers 1 and 2; and 2 and 3 were found to be statistically significant. As analysis of the male

sample was undertaken after that of the female sample, the absence of a statistically significant degree of variation in the assignment of TPS by observers 1 and 3 may indicate that for these observers, who represent the lowest and highest levels of experience in radiographic interpretation respectively, experience in the application of the method did not significantly influence their evaluation of the epiphyseal scar. In contrast, as both interactions involving observer 2 were found to exhibit statistically significant degrees of variation, it may be suggested that experience in the application of the method altered the evaluation of the epiphyseal scar by this individual,

In both female and male cohorts, the lowest percentage agreement occurred in the interactions involving the observer with the least experience in radiographic interpretation. As all observers included in the inter-observer testing had the same level of experience in the application of the method, the influence that this may have on the consistency of the responses is negligible. Consequently, this result suggests that experience in the interpretation of radiographic images rather than in the application of the method may be the determining factor in the consistency of TPS values assigned by multiple assessors.

The results of this analysis suggested that there was a statistically significant relationship between observer and TPS; however the inclusion of sex as an explanatory variable rendered this interaction statistically insignificant. These results suggest that while variation between the TPS assigned by different observers may exist, the influence that the interpretation of the observer has on the assignment of TPS is not statistically significant. This study therefore suggests that the scoring system developed in this study is reliable and repeatable when applied by multiple observers.

4.3.2 Discussion of the overall persistence of the epiphyseal scar in the proximal humerus

Unlike many other regions of the skeleton, there is a paucity of radiographic studies which utilise the development and maturation of the proximal humerus in age estimation; although alternative imaging modalities and dry bone analyses have been employed in age estimation from this region (Zydek *et al.*, 2011). In juvenile individuals, an assessment of age may be undertaken based on the stage of

ossification and fusion of the proximal epiphysis of the humerus which, through its formation from multiple centres of ossification, presents a significant quantity of information from which an assessment of skeletal age may be undertaken (Scheuer and Black, 2000). As a result of the omission of the proximal humerus from the body of radiographic age assessment methods, limited reference has been made to the proximal humerus in respect of the epiphyseal scar (Hall and Rosser, 1963; Acsadi and Nemeskeri, 1970; MacLaughlin, 1987). Despite this, persistent epiphyseal scars have been noted as an additional feature in adult age estimation studies (Acsadi and Nemeskeri, 1970; Workshop of European Anthropologists, 1980). Methods of age estimation from the proximal humerus, such as that by Acsadi and Nemeskeri (1970) include an approach based on the age related expansion of the medullary cavity and the progressive loss of cancellous bone within the humeral head. Age related expansion of the medullary cavity may extend to the region of the epiphyseal scar, however it has been reported that the epiphyseal scar may remain a prominent feature (Hall and Rosser, 1963).

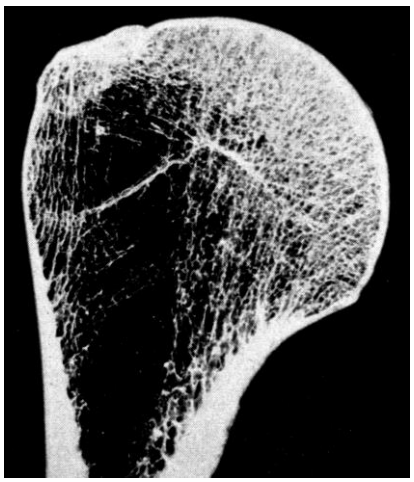

Figure 4.5: Persistence epiphyseal scar in the proximal humerus adapted from Hall and Rosser 1963

Despite the observations of Hall and Rosser (1963), no attempt has been made to examine the proportion of the adult population in whom this feature remains. A study by Klenerman (1969) stated that the persistent epiphyseal scar was observed in all individuals included in their sample. Although the chronological ages of the individuals included in this study was not known, it was stated that encroachment of the medullary cavity on the epiphyseal scar occurred (Klenerman, 1969). It is therefore inferred that the individuals included in this

study were older adults (Hall and Rosser, 1963; Acsadi and Nemeskeri, 1970). These studies suggest that the epiphyseal scar in the proximal humerus has been acknowledged as a persistent feature in adult individuals, despite age related alterations to the surrounding cancellous structure.

This study found that some remnant of an epiphyseal scar persisted in 94.1% of females and 94.82% of males, however no individuals were observed to retain a radiographically identifiable complete epiphyseal scar in either sex cohort. It should be considered however that the degree of persistence observed from a clinical radiographic examination may differ from that observed through the inspection of a radiographic image of dry-bone, such as Figure 4.5, or a section of dry bone. The maximum TPS value observed in the proximal humerus was 9. This was assigned to a single female individual. Although the presence of a single TPS value of 9 cannot be used to infer any pattern, the absence of high persistence scores suggests that within the proximal humerus, complete retention of the epiphyseal scar is unlikely to be observed through clinical imaging of a living individual of either sex.

As the obliteration of the epiphyseal scar has predominantly been associated with alteration to the underlying cancellous structure through the continuous process of remodelling, it was necessary to first assess the relationship between chronological age and the assignment of TPS as a function of the level of obliteration (O'Connor *et al.*, 2008). Initial assessment of the relationship between chronological age and the persistence of the epiphyseal scar suggested that an inverse relationship between mean chronological age and TPS occurred in both female and male individuals; with the strength of the relationship found to be stronger in females than males. As no individuals were observed to retain a complete epiphyseal scar, it is suggested that remodelling of this feature at the proximal humerus is likely to occur in at least one-third of the bone from an early age, i.e. soon after the completion of epiphyseal fusion.

Subsequent analyses suggested that complete obliteration of the epiphyseal scar (score 0) was unlikely to occur in females of 22 years or younger or males of 24 years or younger. The results of linear regression analyses suggested that there

was a positive relationship between the chronological age of an individual and the persistence of the epiphyseal scar; with the strength of the relationship greater in the male sample than in the female sample. This result suggests that chronological age exerts a greater influence on the obliteration of the epiphyseal scar in males than females.

Analysis of the statistical relationship between the persistence of the epiphyseal scar in the proximal humerus and chronological age, biological sex and side of the body showed that when considered as independent variables, only chronological age exhibited a statistically significant relationship with persistence of the epiphyseal scar. Although statistically significant, this relationship explained only 2.5% of variation in TPS value. The inclusion of biological sex within the explanatory model resulted in an increase in the coefficient of determination, however this interaction was not found to be statistically significant. These results suggest that the persistence of the epiphyseal scar in the proximal humerus is predominantly influenced by factors other than those considered by this study. Consequently, it is necessary to consider the results in the light of the wider context and variation which may account for the discrepancies in the assignment of TPS.

The shoulder joint is a highly complex structure, through which the proximal humerus is exposed to a large number of forces including those generated by the muscles of the thorax, upper limb and back (Högfors *et al.*, 1987; Karlsson and Peterson, 1992; Terry and Chopp, 2000). Through its articulation with the glenoid fossa of the scapula, the humeral head represents the sole osseous connection between the arm and the trunk (Standring, 2008). As such, any forces to which the upper limb is exposed must be transmitted through the head of the humerus or surrounding soft tissue. Due to the influence of mechanical stimulation in the rate of osseous remodelling, the exposure of the proximal humerus to external loading may result in an alteration to the rate of bone turnover within this region (Frost, 1996; Frost *et al.*, 1998; Skerry, 2006). As a result of the potential influence of extrinsic and intrinsic forces on the persistence of the epiphyseal scar in the proximal humerus, it was necessary to ascertain the nature of these forces and the region of the bone to which the force is applied.

The proximal humerus, unlike the femur, is not under continuous axial loading, with the exception of the forces applied by gravity and the counter-acting muscular action. As a result, the forces to which the proximal humerus is subjected result from the direct action of the muscles that take their origin from, or insert into, the proximal end of the humerus and the intrinsic forces of the glenohumeral joint and the associated joint capsule (DeFranco and Cole, 2009). Consequently, it is necessary to consider the factors that may lead to variation within these forces in an attempt to discern their ultimate effect on the persistence of the epiphyseal scar.

Male individuals generally exhibit a larger muscle mass than females, particularly in the upper limb (Abe *et al.*, 2003; Wells, 2007). This is primarily attributable to the higher levels of testosterone to which males are exposed and the longer duration of their developmental phase (Wells, 2007). The variation which may exist in the muscle mass of individuals at the proximal humerus is inextricably linked to physical activity and associated strength requirements and may result in significant intra-sex variation (Hunter *et al.*, 2000). From the results derived from the analysis of the persistence of the epiphyseal scar in the proximal humerus and the data presented within the literature relating to the variation in muscle mass in the shoulder region, it is hypothesised that a dominant factor in determining the pattern of obliteration of the epiphyseal scar in the proximal humerus may be due to the stimulation of bone remodelling through the application of mechanical force and the process of cellular mechanotransduction (see section 1.4.2.1). The applied load may be generated by the action of the musculature surrounding the shoulder joint complex, including those of the arm, anterior chest wall and the rotator cuff (Hunter *et al.*, 2000; Abe *et al.*, 2003; Wells, 2007). To investigate this effect further, radiographs of the proximal humerus were examined in three discrete areas and the degree of variation in the persistence of the epiphyseal scar.

4.3.3 Discussion of the regional variation in the persistence of the epiphyseal scar within the proximal humerus

It is hypothesised that an increase in mechanical loading may result in an increase in bone remodelling and consequently an increase in the obliteration of the epiphyseal scar. The results of this study show that the greatest persistence of the

epiphyseal scar in the proximal humerus occurs within the central third of the bone in both sex cohorts. The lowest mean persistence score was assigned to the medial third of the proximal humerus in both the female and male sample cohorts. The variation between the persistence scores assigned to females and males in the medial third of the proximal humerus was not statistically significant. This indicates that remodelling of the epiphyseal scar within this region may be influenced by similar factors in both sexes. It is hypothesised that the application of force may be the primary driver through which remodelling and obliteration of the epiphyseal scar occurs. Consequently, the force applied to the articular surface of the humeral head through intracapsular loading may result in a similar rate of remodelling in females and males.

As the medial third of the humeral head, as designated by this study, is contained within the glenohumeral joint capsule and as a result does not form a site for the origin or insertion for the surrounding musculature, it is concluded that the dominant force to which this region is exposed will likely be intrinsic to the joint capsule. The intracapsular force generated at the glenohumeral joint has been reported to approach the force generated by body weight when the arm is placed in 90° abduction, (Högfors *et al.*, 1987). Although the body mass of males is generally greater than females, the maximum diameter of the humeral head is also larger, resulting in a greater articular surface area over which the intracapsular force is distributed (Krogman, 1962; Stewart, 1979; Bass, 2005). This may account for the variation in force generated and therefore the influence of intracapsular loading on the remodelling rate within the medial aspect of the humeral head. A study by Hashimoto *et al*. (1995) found that the intracapsular force ranged between approximately -25 mmHg and approximately -120mmHg at arm to trunk angles of 80° and 180° respectively. The effect of the position of the humeral head on intra-articular pressure was also noted by Yamamoto *et al*. (2006). It has also been shown that the load applied to the limb may influence the intra-articular pressure, with an increase in load of 1kg resulting in a 5-fold increase in the mean intracapsular negative pressure (Yamamoto *et al.*, 2006). This variation in the pressure to which the medial aspect of the humeral head is exposed may result in an alteration to the rate at which remodelling takes place, depending on the

frequency with which the arm is placed in a position conducive to the generation of maximum force (Frost, 1996; Skerry, 2006). As the movement through which this increase in intracapsular joint force is not dependent on the sex of the individual, it is hypothesised that the rate of remodelling within this region may be similar in both sexes. This hypothesis was supported by the similarities in remodelling between females and males in the medial third of the bone observed in this study.

Although the lateral region was observed to exhibit intermediate levels of persistence of the epiphyseal scar in relation to the central and medial thirds of the proximal humerus, this was the only site at which a statistically significant difference was found between the persistence scores assigned to females and males. This was also the only region of the proximal humerus where the mean persistence score was observed to be greater in females than males. Within this region, it was also noted that males were more likely than females to be assigned a score of 0 or 1; however the reverse was true for persistence scores 2 or 3. These results suggest that within this region of the proximal humerus, female individuals are more likely to retain a greater proportion of the epiphyseal scar than males. The lateral aspect of the proximal humerus, while not influenced by intracapsular forces, forms an attachment site for the powerful muscles of the rotator cuff (RC), three of which (supraspinatus, infraspinatus and teres minor) insert into the greater tubercle with the fourth muscle, subscapularis, inserting into the lesser tubercle (Figure 4.6) (Terry and Chopp, 2000).

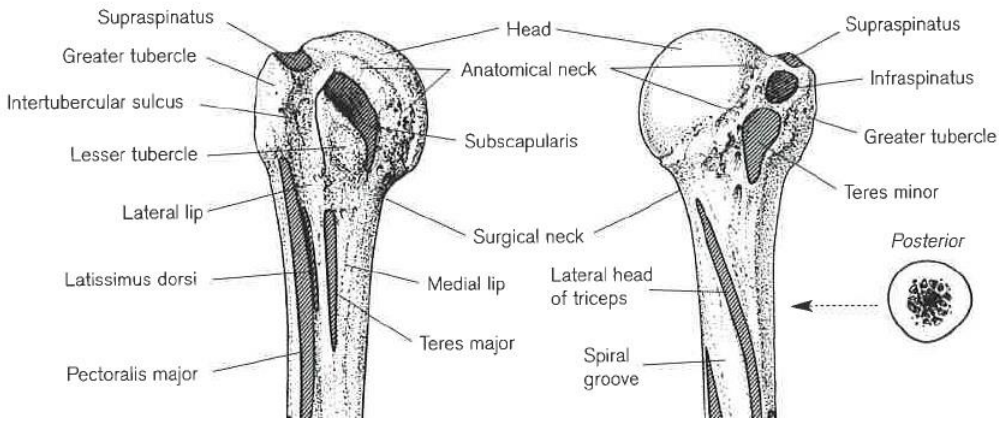

Figure 4.6: Muscular attachment sites of the proximal humerus. Adapted from Scheuer and Black (2000)

As a result of these attachments, the lateral third of the humeral head is exposed to forces generated through the contraction of these muscles, transmitted through their tendinous insertions (DeFranco and Cole, 2009). As male individuals generally exhibit a larger muscle mass than females, particularly in the upper limb, this result supports the hypothesis that stimulation of bone turnover by mechanical loads generated by the muscles of the RC may result in an increased obliteration of the epiphyseal scar (Gallagher *et al.*, 1997). This is supported by the literature which states that in individuals with complete RC tearing there is an increased risk of osteopenia as a result of reduced mechanical stimulation of bone formation (Jiang *et al.*, 2002; Meyer *et al.*, 2004; Cadet *et al.*, 2008; DeFranco and Cole, 2009). As a reduction in bone mass to osteopenic levels requires the loss of cancellous bone surface area, it is reasonable to hypothesise that the rate of remodelling within an osteopenic individual will be lower than that of a healthy individual within the same anatomical region. Consequently, the reduction in bone mass observed in individuals suffering from complete RC tearing suggests that in individuals without injury, there will be a greater rate of remodelling to maintain structurally competent cancellous bone (DeFranco and Cole, 2009). From this it can be inferred that the action of the rotator cuff stimulates bone formation, which as previously reported, is coupled with bone resorption, resulting in an increase in bone turnover. This may in turn induce a reduction in the persistence of the epiphyseal scar within the lateral third of the bone.

The greatest mean persistence of the epiphyseal scar in the proximal humerus was observed in the central third in both females and males. Although a greater mean persistence rate was found in the male sample than the female sample, the highest persistence of an epiphyseal scar was noted in a single female individual, where a persistence score of 4 was observed. This result indicates that it is unlikely that the epiphyseal scar will remain intact within the central third of the proximal humerus in either sex, but will persist as a partial or fenestrated structure. The majority of individuals in both the female and male samples were assigned a persistence score of 2. This suggests that it is most likely that an individual will retain at least 50% of the scar within the central third of the proximal humerus. Although inter-sex variation was observed in the persistence of the epiphyseal scar

in this region, the results of an analysis of variance suggested that there was no significant difference between the persistence of the epiphyseal scar in females and males in this region of the proximal humerus.

Although the ligaments that form the joint capsule insert into the anatomical neck of the proximal humerus, which lies largely within the central region of the bone as considered by this study, tension is only applied when the arm has reached the extremes of its motion i.e. extreme abduction/adduction as a result of its primary purpose which is to prevent translocation of the humeral head (Terry and Chopp, 2000; Standring, 2008). Consequently, loading may be applied transiently and infrequently and therefore may not influence the rate of bone turnover within the central region of the bone to a significant degree. This is supported by the similarities observed between females and males in this study. It is therefore suggested that any obliteration of the epiphyseal scar that occurs within the central third of the proximal humerus is as a result of bone turnover that occurs at a slower rate than in either the medial or lateral regions of the bone. It is important to note that the observation of an epiphyseal scar from a radiographic image represents a two-dimensional representation of a three-dimensional object (Cotti and Campisi, 2004; Jennane *et al.*, 2007). Consequently, image superimposition may alter the appearance of an epiphyseal scar.

Initial analysis of the variation between persistence scores assigned to the medial, central and lateral regions of the proximal humerus suggested that there was a statistically significant variation between all regions in both female and male cohorts. Although discrete analyses suggested that the variation between males and females within each region was only statistically significant in the lateral third of the bone, this study suggested that the variation in persistence score attributable to biological sex was not statistically significant overall. Further analyses found that there was a statistically significant relationship between chronological age and persistence of the epiphyseal scar within these discrete regions however only 1.6% of variation in TPS was attributable to variation in age. In addition to chronological age, the results of a GLM analysis found that there was a statistically significant relationship between region of the bone and the persistence of the epiphyseal scar. This analysis also found that variation in the

region of the bone explained 24.7% of the variation in the persistence of the epiphyseal scar. The strongest explanatory model for the persistence of the epiphyseal scar was found to be region and biological sex, which explained 25.5% of variation in the assigned persistence score.

The results obtained from the analysis of the persistence of the epiphyseal scar within three discrete regions suggest that the overall variation in the epiphyseal scar is largely due to variation within the lateral third of the proximal humerus. These results also suggest that the greatest degree of obliteration of the epiphyseal scar is likely to occur within the medial third of the bone. The findings of this study therefore support the hypothesis that the obliteration of the epiphyseal scar is likely to be under greater influence from the application of force than from senescent alteration to bone.

As a statistically significant inter-sex variation was observed only in the lateral third of the bone, this study suggests that muscular loading of the lateral third of the proximal humerus may be one of the primary drivers of osseous remodelling of the epiphyseal scar in this region. Within the medial region, the absence of a statistically significant difference between the persistence of the epiphyseal scar in females and males suggests that there may be a function related driver of remodelling and therefore obliteration of the epiphyseal scar.

5 Persistence of the epiphyseal scar in the distal radius

5.1 Sample Distribution

The sample distribution according to age, sex and side of the body are presented in Table 5.1.

Table 5.1: Distribution of the sample used in the analysis of the distal radius according to chronological age, biological sex and side of the body

Age	Female Right	Female Left	Male Right	Male Left
20	5	5	5	5
21	5	5	5	5
22	5	5	5	5
23	5	5	5	5
24	5	5	5	5
25	5	5	5	5
26	5	5	5	5
27	5	5	5	5
28	5	5	5	5
29	5	5	5	5
30	5	5	5	5
31	5	5	5	5
32	5	3	5	5
33	5	5	5	5
34	5	5	5	5
35	5	5	5	5
36	5	5	5	5
37	5	5	5	5
38	5	5	5	5
39	5	5	5	5
40	5	5	5	5
41	5	5	5	5
42	5	5	5	5
43	5	5	5	5
44	5	5	5	3
45	5	5	5	5
46	5	5	5	5
47	5	5	5	5
48	5	5	5	5
49	5	5	5	5
50	5	5	5	5
Total	**155**	**153**	**155**	**153**

5.2 Results

5.2.1 Intra-Observer Analysis

Initially, a series of ANOVA were undertaken to assess the statistical significance of the variation in the assignment of TPS by a single observer on multiple occasions. These analyses suggested that there was no significant difference between the TPS assigned to the female (P=0.847) or male (P=0.112) groups at the first or second attempt.

Analysis of the data obtained from intra-observer assessments showed that 80% of TPS values assigned to females and 76.67% of TPS values assigned to males were within two scores of those assigned during the first round of assessment. The variation in TPS between the first and second rounds of assessment according to sex is presented in Figure 5.1.

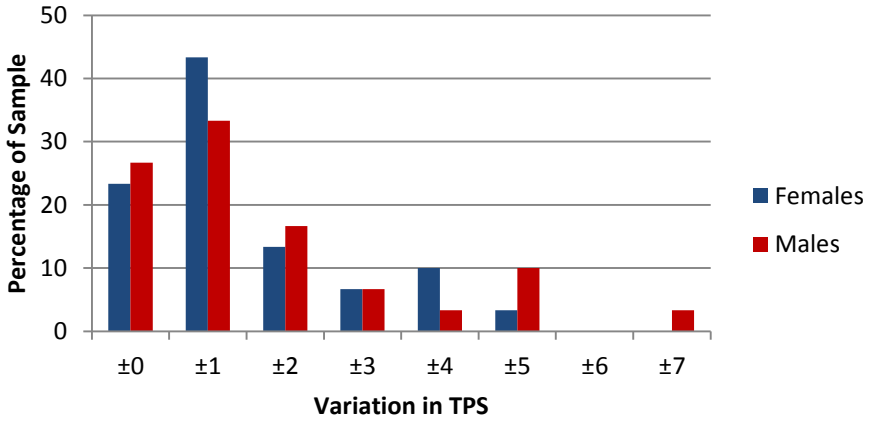

Figure 5.1: Intra-observer variation in Total persistence Score assigned to the distal radius according to biological sex

The data presented in Figure 5.1 showed that the maximum variation between assigned TPS values was ±7. This degree of divergence between scores was observed in 3.33% of the male sample.

To assess the statistical relationship between rounds of assessment, biological sex and the assignment of TPS, a series of GLM analyses were undertaken, the results of which are presented in Table 5.2.

Table 5.2: Results of the General Linear Model analysis of the intra-observer variation in the distal radius

Factor(s)	P-Value	R^2	Adjusted R^2	Percentage Variation
Sex	0.456	0.005	-0.004	0%
Round	0.197	0.014	0.006	0.6%
Sex*Round	0.308	0.028	0.002	0.2%

These results suggest that although the round of assessment explained the greatest degree of variation in TPS (R^2=0.006), the relationship was not statistically significant (P=0.197). These analyses also supported the earlier findings which suggested that inter-sex variation in TPS was not statistically significant (P=0.456). The relationship between these factors when combined was not found to exhibit a statistically significant relationship with TPS (P=0.308).

The results of the analyses undertaken to assess the intra-observer consistency in the assignment of TPS suggest that the method is repeatable when applied in the distal radius by a single observer on multiple occasions.

5.2.2 Inter-Observer Analysis

Initial analysis of the data resulting from the inter-observer test of the method in the distal radius was undertaken through a series of one-way ANOVA. The results of these analyses suggested that the variation observed within the TPS values assigned to female individuals by three observers was not statistically significant (P=0.054). In contrast, the variation observed within the TPS values assigned to male individuals by three observers was statistically significant (P=0.048).

The percentage agreement between pairs of observers was calculated for both the female and male sample. These results, presented in Table 5.3, suggest that the greatest percentage agreement was found between observers 1 and 2 in both sex samples. Within the female sample, the lowest percentage agreement was found between observers 1 and 3 while in the male sample, this was found between observers 2 and 3. Across all pair-wise comparisons, inter-observer agreement was greater in the male sample than the female sample.

Table 5.3: Inter-observer percentage agreement in Total persistence Score in the distal radius

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	86.67	63.33	66.67
Male	93.33	83.33	76.67

To assess the statistical significance of the inter-observer variation in the assignment of TPS values, a series of one-way ANOVA were conducted. The results of these analyses, presented in Table 5.4, showed that in both sex cohorts, the variation in the data obtained from observers 2 and 3 exhibited the highest degree of statistical significance. In both females and males, the variation between the TPS values assigned by observers 1 and 2 was not statistically significant. As the only set of observer interactions in which this occurred, these data indicate that the data obtained from observer 3 were significantly different to those obtained from either of the remaining participants.

Table 5.4: Statistical significance of inter-observer variation in the assignment of Total Persistence Scores in the distal radius according to biological sex

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	0.511	0.067	0.024
Male	0.753	0.043	0.027

To examine the statistical relationship between observer and assignment of TPS further, a series GLM analyses were undertaken, the results of which are presented in Table 5.5.

Table 5.5: Results of the General Linear Model analysis of inter-observer variation in the distal radius

Factor(s)	P-Value	R^2	Adjusted R^2	Percentage Variation
Sex	0.234	0.008	0.002	0.2
Observer	0.007	0.054	0.044	4.4
Observer * Sex	0.934	0.063	0.036	3.6

The results of these analyses suggested that there was no statistically significant relationship between biological sex and the assignment of TPS in the distal radius (P=0.234). Analysis of the relationship between observer and the assignment of TPS suggested that there was a statistically significant interaction between these

factors (P=0.007) and that variation in observer accounted for 4.4% of variation in TPS. As the combined interaction of biological sex and observer on TPS was not found to be statistically significant (P=0.934), these results suggest that variation in observer represents the best explanatory model for the assignment of TPS in the inter-observer assessment. As these analyses were conducted using individuals of both sexes however, these results suggest the method is statistically repeatable when applied by multiple observers.

5.2.3 Main Data Analysis

Initial analysis was undertaken to determine the normality of the distribution of the data derived from the assessment of radii from the female and male samples. The results suggested that the distribution of the data according to TPS was not statistically normal in either the female (W-statistic=0.956; P=<0.001) or male (W-statistic=0.930; P=<0.001) data sets. The distributions of the data for female and male cohorts are presented in Figure 5.2.

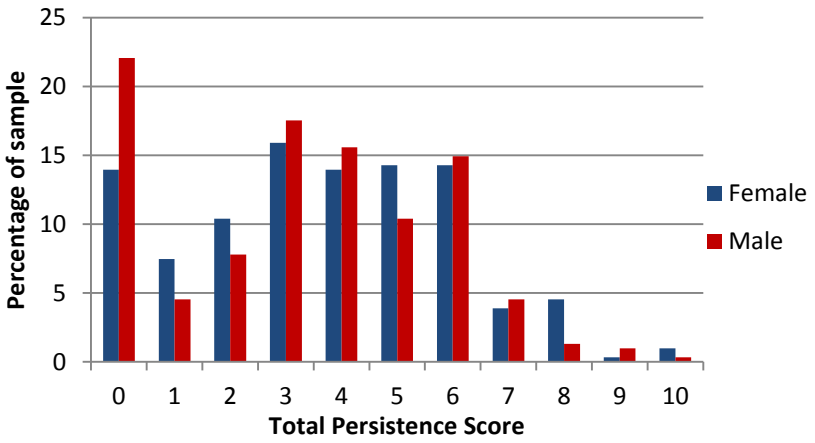

Figure 5.2: Distribution of the distal radius study sample according to biological sex and Total Persistence Score

These results suggested that 86.04% of females and 77.92% of males retained some remnants of the epiphyseal scar. A one-way ANOVA was conducted to assess the statistical significance of the variation in the assignment of TPS between females and males, the results of which determined that any variation present was not statistically significant (P=0.100). Within both sex cohorts, the maximum TPS value assigned was 10 (out of a possible maximum of 12). As no individuals were assigned a score of 12, these results showed that no subjects were observed to retain a complete epiphyseal scar in this anatomical region. Although some

individuals were assigned TPS values of greater than 6, the majority of subjects included in this study were observed to fall within the TPS 0-6 range. The highest percentage of individuals represented by a single TPS value was found in the male cohort of individuals to whom a TPS value of 0 had been assigned, which represented 22.08% of the male sample population.

To assess the relationship between chronological age and TPS, the mean chronological age for the individuals assigned to each TPS was calculated. These results are presented in Table 5.6 and Table 5.7 for females and males respectively.

Table 5.6: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal radius in female individuals

Total Persistence Score	Mean (years)	Maximum (years)	Minimum (year)
0	35.03 (n=68)	50	20
1	39.86 (n=14)	50	20
2	38.21 (n=24)	50	20
3	34.09 (n=54)	50	22
4	32.98 (n=48)	50	20
5	30.72 (n=32)	50	20
6	36.15 (n=46)	50	20
7	37.86 (n=14)	50	24
8	44.75 (n=4)	50	20
9	33.67 (n=3)	38	38
10	25.00 (n=1)	41	23
11	--		
12	--		

Table 5.7: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal radius in male individuals

Total Persistence Score	Mean (years)	Maximum (years)	Minimum (years)
0	35.65 (n=43)	50	20
1	37.61 (n=23)	50	25
2	35.31 (n=32)	50	27
3	34.16 (n=49)	49	20
4	32.37 (n=43)	49	20
5	38.11 (n=44)	46	20
6	34.05 (n=44)	49	20
7	36.75 (n=12)	50	20
8	31.79 (n=14)	50	37
9	38.00 (n=1)	40	29
10	32.33 (n=3)	25	25
11	--		
12	--		

As TPS values represent a scale against which the mean chronological age of individuals represented by each cohort may be measured, the net difference in mean chronological age between cohorts 1 and 7 was calculated for both sexes. These TPS values were selected as they represent the highest TPS value, where n>10, common to both sexes. In both females (-2 years) and males (-0.86 years), this calculation resulted in a negative value. Consequently, these data suggest that there may be an inverse relationship between mean chronological age and increasing TPS in the distal radius.

The relationship between chronological age and the maximum and minimum TPS values was examined by calculating the percentage of individuals within each one-year cohort represented by TPS 0 or TPS≥9. A linear regression analysis was conducted to assess the strength of the relationship between these values. The results of these analyses are presented in Figure 5.3 and Figure 5.4 for females and males respectively.

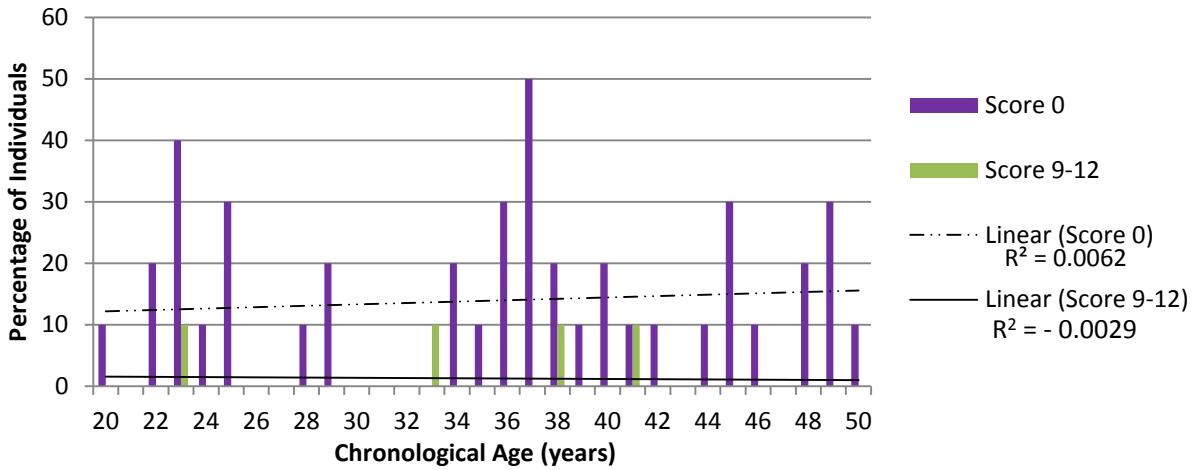

Figure 5.3: Percentage of female individuals exhibiting complete obliteration and maximum persistence of the epiphyseal scar in the distal radius according to chronological age

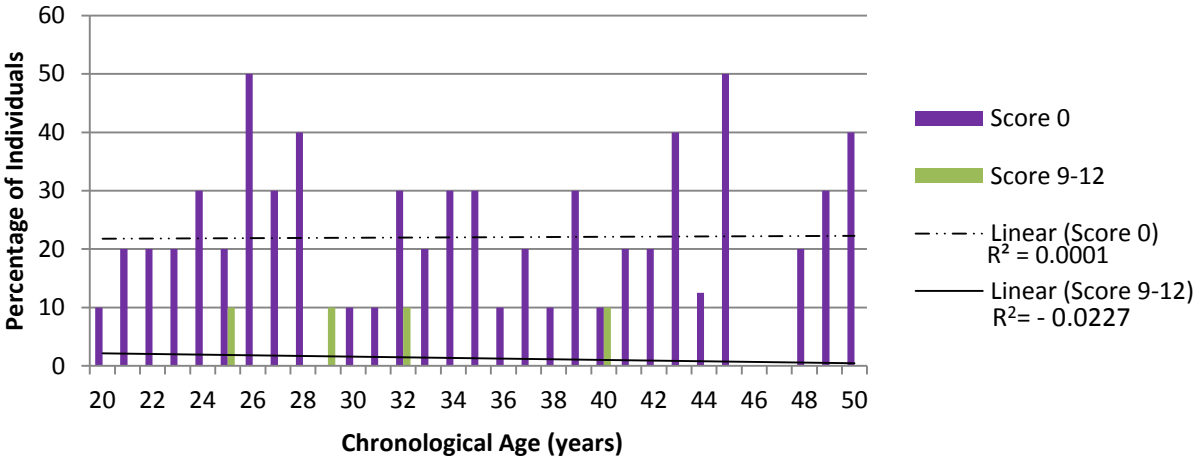

Figure 5.4: Percentage of male individuals exhibiting complete obliteration and maximum persistence of the epiphyseal scar in the distal radius according to chronological age

The results of these analyses suggest that there is a weak positive trend in the percentage of individuals to whom a TPS value of 0 was assigned in both the female (R^2=0.0062) and male (R^2=0.0001) cohorts, although this trend appeared to be marginally stronger in females than males. A weak negative trend in the percentage of individuals to whom a TPS value ≥9 was assigned was observed in both the female (R^2=- 0.0029) and male (R^2= - 0.0227) samples, however this relationship was marginally stronger in males than females. These results suggest that complete obliteration of the epiphyseal scar and obliteration of up to one-third of the epiphyseal scar occurs largely independently of chronological age in both sexes. Consequently, the relationship between TPS and other biological characteristics was considered and the variation attributable to these factors quantified through the application of a GLM analysis. This analysis facilitated the quantification of the variation explained by chronological age in addition to the factors of biological sex and side of the body. The results of the GLM analysis are presented in Table 5.8.

Table 5.8: Results of the General Linear Model analysis in the distal radius

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.190	0.059	0.011	1.1%
Sex	0.072	0.005	0.004	0.4%
Side	0.684	0.000	-0.001	-0.1%
Age*sex	0.099	0.0129	0.033	3.3%
Age*side	0.559	0.105	0.007	0.7%
Sex*side	0.198	0.008	0.003	0.3%
Age*sex*side	0.587	0.220	0.025	2.5%

The results of this analysis showed that although chronological age appears to explain the greatest degree of variation in TPS of any independent factor, this variable was not found to exhibit a statistically significant relationship with TPS value (P=0.190). Through this analysis, it was also found that there was no statistically significant relationship between sex (P=0.072) or side of the body (P=0.684) and TPS value. The interaction between age and sex was found to explain the highest degree of variation in TPS (R^2=0.033); however this relationship was not statistically significant (P=0.099). The results of the GLM analyses presented in Table 5.8 suggest that in the distal radius, the factors assessed in this study do not exert a statistically significant influence on TPS and therefore persistence of the epiphyseal scar either as independent or co-dependent variables.

Although not statistically significant, it was deemed appropriate to consider the potential influence of limb dominance on the persistence of the epiphyseal scar as a function of greater mechanical loading in the preferred side. A series of one-way ANOVA were conducted to assess the statistical significance of any variation between left and right sides of the body within single-sex cohorts and between sex cohorts. The results of these analyses are presented in Table 5.9.

Table 5.9: Reciprocal table of Analyses of Variance results for limb laterality tests in the distal radius according to biological sex

	Female Left	**Male Right**
Female Right	0.288	0.044
Male Left	0.739	0.536

To address the potential masking effect of examining the scar as a single entity, the variation in the persistence of the epiphyseal scar within three discrete regions of the distal radius was calculated. Initially, the mean regional persistence scores for the medial, central and lateral thirds of the distal radius were calculated. The resulting data are presented in Table 5.10.

Table 5.10: Mean regional persistence scores for females and males in the distal radius

	Medial Region	**Central Region**	**Lateral Region**
Female	1.08	1.41	1.17
Male	1.09	1.26	0.95

These data show that, with the exception of the medial third of the distal radius, higher mean RPS values were achieved in females relative to males. In both sex cohorts, the highest mean RPS value was observed in the central region; however the position of the lowest mean RPS value differed between sexes. In females, this was found to occur in the medial region, while in males, the lowest mean persistence of the epiphyseal scar was observed in the lateral region.

To examine the distribution of persistence scores within each of the regions of the distal radius in greater detail, the percentage of individuals to whom each regional persistence score was assigned in each section of the bone was calculated. The resulting data are presented in Table 5.11 and Table 5.12 for females and males respectively.

Table 5.11: Percentage distribution of Regional Persistence Scores in the distal radius in female individuals

Persistence Score	Medial Region	Central Region	Lateral Region
0	35.39	25.65	30.84
1	27.27	24.68	26.30
2	31.82	37.01	38.64
3	5.19	8.77	3.57
4	0.32	3.90	0.65

The greatest percentage of individuals to whom RPS values of 0 or 1 were assigned occurred in the medial and lateral thirds for females and males respectively. In females, 62.66% of individuals were assigned an RPS value ≤1 in the medial third, while in males 62.99% of individuals were represented by this cohort in the lateral third of the distal radius.

Table 5.12: Percentage distribution of Regional Persistence Scores in the distal radius in male individuals

Persistence Score	Medial Region	Central Region	Lateral Region
0	36.04	30.19	43.51
1	22.73	21.43	19.48
2	37.66	42.21	35.06
3	3.25	4.22	1.95
4	0.32	1.95	0.00

The greatest percentage of individuals to whom persistence scores of 2 or 3 were assigned occurred in the central third of the bone in both females and males,

where 45.78% and 46.43% of individuals were represented by this cohort respectively. Similarly, in both sexes, the highest percentage of individuals to whom maximum persistence of the epiphyseal scar (RPS 4) was assigned was found in the central third of the bone. In the medial third of the bone, the percentage of individuals represented by this RPS value was equivalent in females and males, however in both the central and lateral thirds of the bone, a higher percentage of females were found to exhibit maximum persistence of the epiphyseal scar.

In addition to the calculation of the percentage representation of the cohort by each persistence score, the statistical significance of the variation in persistence scores between regions of the bone were calculated using a series of one-way ANOVA, the results of which are presented in Table 5.13.

Table 5.13: Statistical significance of the inter-region variation in regional persistence scores in the distal radius according to biological sex

	Medial v Central	Central v Lateral	Lateral v Medial
Female	<0.001	0.012	0.201
Male	0.043	<0.001	0.081

The results of these analyses showed that in the female cohort, statistically significant degrees of variation in RPS values were present between the medial and central regions; and the central and lateral regions of the distal radius. A similar pattern of statistically significant variation between the regional persistence of the epiphyseal scar occurred in the male sample. In contrast, however, to the female cohort, the highest statistically significant variation was observed between the central and lateral thirds of the bone. No statistically significant difference occurred between the lateral and medial thirds of the distal radius in either females or males.

To assess the influence of chronological age, biological sex and side of the body on the persistence of the epiphyseal scar within these discrete regions of the bone, a series of GLM analyses were conducted, the results of which are presented in Table 5.14.

Table 5.14: Results of the General Linear Model analyses for regional variation in the persistence of the epiphyseal scar in the distal radius

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	<0.001	0.038	0.022	2.2
Sex	0.012	0.003	0.003	0.3
Side	0.571	0.000	0.000	0
Region	<0.001	0.016	0.015	1.5
Age*sex	<0.001	0.083	0.052	5.2
Age*side	0.003	0.068	0.036	3.6
Sex*side	0.072	0.005	0.004	0.4
Region*side	0.915	0.016	0.013	1.3
Region*sex	0.112	0.021	0.019	1.9
Region*age	0.998	0.071	0.023	2.3
Age*sex*side	0.002	0.142	0.081	8.1
Region*side*sex	0.978	0.024	0.018	1.8
Region*side*age	0.994	0.120	0.022	2.2
Region*sex*age	0.992	0.138	0.042	4.2
Region*sex*age*side	1.000	0.229	0.035	3.5

Contrary to the results obtained from the analysis of the epiphyseal scar as a whole, the results of the regional GLM analyses suggest that a statistically significant relationship exists between chronological age and persistence score ($P<0.001$). This relationship was found to explain 2.2% of the variation in regional persistence score. Statistically significant relationships were also observed between persistence score and sex ($P=0.012$); and region ($P<0.001$). These relationships were found to explain 0.3% and 1.5% of variation in regional persistence of the epiphyseal scar respectively. Through the application of further analyses, it was observed that the interaction between chronological age, biological sex and side of the body produced the greatest explanatory model in relation to persistence score. This interaction was found to be statistically significant ($P=0.002$) and explained 8.1% of the variation in persistence of the epiphyseal scar within the discrete regions of the bone. Despite being statistically significant when considered independently, region of the bone was not included in any further statistically significant interactions.

5.3 Discussion of the persistence of the epiphyseal scar in the distal radius

5.3.1 Discussion of intra-observer and inter-observer analysis in the distal radius

This study found that the variation between TPS values assigned by a single observer on two occasions was not statistically significant in either female or male individuals; however there was a greater degree of intra-observer agreement within the female sample than the male sample. Further analyses found that there was no statistically significant relationship between persistence of the epiphyseal scar and round of assessment when considered as an independent variable or as a co-variable with biological sex. These results, combined with those of the initial analyses, suggest that the staging system presented in this study may be applied consistently to the distal radius by a single individual on multiple occasions.

Within the female sample, 80% of TPS values assigned at the second attempt were within two scores of those assigned during the first round of assessment. In male individuals, the percentage intra-observer agreement decreased to 76.67%. As assessment of the female sample was undertaken prior to that of the male sample, these results may indicate that experience in the application of the scoring system to the distal radius may not affect the level of intra-observer consistency achieved in this anatomical region.

In addition to the level of intra-observer agreement, it was prudent to examine the level of variation in the assignment of TPS values between multiple observers. This study found that the TPS values assigned by the three observers did not differ significantly in the female sample; however a statistically significant degree of variation was observed within the male sample. Although this provided a foundation on which to base an assessment of inter-observer consistency, further analysis was required to examine the variation between individual pairs of observers. This study found that the greatest percentage agreement was achieved between observers 1 and 2 in both sex cohorts, where 86.67% and 93.33% were attained for females and males respectively.

The lowest percentage agreement was found between observers 1 and 3 in the female cohort and 2 and 3 in the male cohort. All interactions involving data provided by observer 3 exhibited statistically significant levels of variation. As observers 1 and 3 represented the lowest and highest degrees of experience in radiographic interpretation respectively, the inconsistency in the results relating to the effect of experience on inter-observer accuracy suggest that the experience of the individual in radiographic age estimation may not influence the level of inter-observer agreement in this anatomical region.

Comparison of the percentage inter-observer agreement obtained from the analysis of the data derived from the assessment of the female and male samples showed that a higher percentage agreement was achieved in the male sample than the female sample in all observer interactions. As all observers conducted their assessment of the female sample prior to those of the male sample, this may suggest that a degree of experience in the application of the method in the distal radius may be beneficial to the level of inter-observer consistency achieved.

5.3.2 Discussion of the overall persistence of the epiphyseal scar in the distal radius

The distal radius, as a component of the wrist, is included in a large number of methods of age estimation which utilise a variety of techniques including plain film radiography (Greulich and Pyle, 1959; Vignolo *et al.*, 1992; Cameriere *et al.*, 2006; Khan *et al.*, 2009), MRI (Dvorak *et al.*, 2007b; George *et al.*, 2012) and Ultrasound (US) (Mentzel *et al.*, 2005; Khan *et al.*, 2009). The hand and wrist, as a function of the ease with which it can be radiographed forms one component of the triumvirate of images recommended for age estimation in living individuals by the German Working Group on Forensic Age Diagnostics (AGFAD) (Schmeling *et al.*, 2003; Kellinghaus *et al.*, 2010). Consequently, it is imperative that the standards on which these methods are based are developed from appropriate maturity criteria or scoring stages which can be validated through statistical analysis.

Within the commonly applied methods of radiographic age estimation, such as those of Greulich and Pyle (1950; 1959) and Tanner *et al*. (1962; 1975; 2001), reference is made to the epiphyseal scar as a line of increased density which is likely to disappear over time but that may persist in some individuals throughout

their adult lives. In contrast, the radiographic atlas by Thiemann and Nitz (1991) states that the final stage of maturation in the distal radius is reached when the epiphysis is no longer recognisable. It is inferred from this that the epiphyseal scar is considered to disappear. No reference is made to this feature in the digital atlas of Gilsanz and Ratib (2005).

Although the possible persistence of the epiphyseal scar in the distal radius has been noted in the literature, the obliteration of the epiphyseal scar in the distal radius has been employed as a criterion in several methods of skeletal age estimation (Todd, 1937; Greulich and Pyle, 1950; 1959; Schmidt *et al.*, 2008; Baumann *et al.*, 2009). A thorough search of the literature however has failed to uncover any prior studies on which this criterion is based. Consequently, the application of the obliteration of the epiphyseal scar in the distal radius as the final maturity criterion in methods of age assessment may not be appropriate.

Due to the increasing frequency with which age estimation from the wrist is applied in both living and deceased individuals, it was highly desirable to undertake a study of the persistence of the epiphyseal scar in this anatomical region (Schmeling *et al.*, 2003). It has been noted in the literature that the epiphyseal scar in the distal ulna becomes completely obliterated during adolescence (Todd, 1937; Greulich and Pyle, 1959). This was supported by a cursory examination of the radiographs collected for use in this study in which no patent epiphyseal scars were observed in the distal ulna. Consequently, only the distal radius was considered in this study.

Initial analysis of the data derived from the assessment of the epiphyseal scar in the distal radius showed that there was a higher TPR in females than males with some remnant of an epiphyseal scar being recorded in 86.04% of females and 77.92% of males. Further analysis of these data showed that the variation in the assignment of TPS between females and males was not statistically significant. The maximum persistence score assigned to either sex cohort was 10; this result suggests that complete persistence of the epiphyseal scar in the distal radius is unlikely to occur in either sex. Closer examination of the data showed that the majority of individuals in both sex cohorts were assigned TPS values of between 0

and 6, suggesting that in the majority of individuals at least 50% of the epiphyseal scar will be remodelled. The complete absence of an epiphyseal scar was only observed in 13.96% and 22.08% of females and males respectively. This result suggests that complete obliteration of the feature may be more likely in males than females, from which it may be hypothesised that the factors which cause the obliteration of the epiphyseal scar are more prominent in males than females.

As obliteration of the epiphyseal scar has, in the literature, been associated with increasing chronological age, the mean chronological age of the individuals assigned to each persistence score was calculated. These results suggested that there may be an inverse relationship between mean chronological age and increasing TPS value in both male and female individuals. The relationship between chronological age and the complete obliteration and persistence of at least two thirds of the epiphyseal scar was assessed (Table 5.11 and Table 5.12). The percentage of individuals assigned a TPS value of 0 and those assigned TPS value ≥9 were calculated for each single year cohort. The results of these assessments suggest that there is a weak positive relationship between the percentages of individuals in whom no epiphyseal scar was observed and increasing chronological age in both females and males. This relationship was found to be stronger in females than males.

Within the cohort of individuals to whom a TPS value of ≥9 was assigned, a weak negative trend was observed in both the female and male samples. In contrast to the results derived from analysis of the TPS 0 cohorts, the strength of the relationship between the percentage of individuals to whom a TPS≥9 was assigned and chronological age was found to be stronger in males than females. These results suggest that the influence of chronological age on the persistence of the epiphyseal scar in individuals with high levels of retention is greater in males than females. Conversely, the influence of increasing chronological age on the complete obliteration of the epiphyseal scar is greater in females than males. The inferences that can be made from these analyses are limited due to the low R^2 values obtained from linear regression analyses. Consequently, it was necessary to conduct further analysis of the data to assess the overall relationships between TPS and biological sex and side of the body in addition to chronological age.

There was no statistically significant relationship between TPS and any of the factors examined by this study. The highest level of significance was observed in the relationship between biological sex and TPS. This result, although not statistically significant, supports the hypothesis that the factors which exert the greatest influence on the level of persistence of the epiphyseal scar are related to differences in remodelling between males and females. Although the interaction between biological sex and TPS was the closest to being statistically significant, the highest coefficient of determination was obtained from the analysis of the relationship between the combined influence of chronological age and biological sex and TPS. This interaction, however, was found to explain only 3.3% of the overall variation in persistence of the epiphyseal scar in the distal radius. This suggests however that approximately 96.7% of variation in the persistence of the epiphyseal scar of the distal radius is not explained by factors included in this study.

It is reported in the literature that approximately 90% of individuals preferentially use their right upper limb during functional tasks such as writing and opening doors (Porac *et al.*, 1980; Steele and Mays, 1995; Plochocki, 2004). Analysis of the overall trends and relationships suggest that the side of the body on which the examination was conducted was not a source of statistically significant variation in TPS.

As a result of functional dominance, an increase in muscle mass has been reported in the dominant limb relative to the non-dominant limb (Steele, 2000). It has been hypothesised that a discrepancy in mechanical loading between the left and right upper limbs may result in variation in the rate of osseous remodelling between sides of the body, thereby inducing an alteration in the overall morphology of the bone and in particular that of the region of the enthesis concerned (Steele and Mays, 1995; Steele, 2000). It should be noted that studies concerning skeletal asymmetry in the upper limb largely relate to alterations in the overall morphology of cortical rather than cancellous bone (Auerbach and Ruff, 2006; Lazenby *et al.*, 2008; Blackburn, 2011; Ozener, 2012). A study by Lazenby *et al.* (2008) however found that within the second metacarpal there was a marked increase in trabecular number, bone volume fraction and ratio of rod to plate

trabeculae in the right hand compared with the left. As the remains examined by Lazenby *et al*. (2008) were obtained from an archaeological cemetery population, no categorical inferences may be drawn regarding the association between the variation in the structure of trabecular bone between right and left hands and the handedness of the individual. The alterations noted by Lazenby *et al*. (2008) could result in cancellous bone of greater structural integrity and with greater resistive capacity to applied loads and are consistent with the hypothesis that preferential functional loading results in changes to the cancellous and cortical structure. This premise has been contested by the work of authors such as Trinkaus *et al.* (1994) who suggest that due to the dynamism of the forces to which the upper limb is exposed, a degree of fluctuating asymmetry occurs which alters the mechanical and structural capabilities of the upper limb to suit the conditions under which it is temporarily placed. Consequently, the cancellous and cortical structures of the bone encountered at the time of examination reflect the stresses to which the upper limb was exposed at the time rather than a prevailing functional dominance.

As much of the literature relating to fluctuating or directional limb asymmetry is based on archaeological samples, it is necessary to consider the effect of secular change in occupational stresses on the manifestation of limb asymmetries (Ruff and Jones, 1981; Cuk *et al.*, 2001). As a result of the reduction in the segregation of female and male occupations and the increase in sedentary work habits, the extrinsic forces to which the distal radius as a component of the upper limb is exposed may be more similar between females and males in modern populations than in archaeological samples (Charisi *et al.*, 2011). Consequently, the degree to which hypotheses on limb laterality and functional dominance based on archaeological remains can be applied to contemporary populations is limited. This study observed that a statistically significant degree of variation existed between the persistence of epiphyseal scars in the right distal radius of females and males (Table 5.9). As no statistically significant variation was observed between the persistence of the epiphyseal scar in the left distal radius, these results may lend support to the hypothesis of fluctuating asymmetry. The presence of a statistically significant degree of variation in the persistence of the epiphyseal scar in the right distal radius between females and males may suggest

that the effect of asymmetry is enhanced by sex-specific characteristics. The results of the GLM model for the combined influence of biological sex and side of the body was not found to exhibit a statistically significant relationship with TPS. These findings may indicate that some of the variation in the persistence of the epiphyseal scar between left and right sides of the body may be explained by the variation observed between males and females.

The results of this study suggest that the rate at which bone remodelling occurs may be largely dependent on factors other than chronological age, biological sex and side of the body. Consequently, it is necessary to consider the potential influences to which the bone is exposed which may result in alteration to the rate of osseous remodelling. According to the theory of functional bone adaptation, the rate and pattern of bone turnover is influenced by the application of a mechanical load. In the context of the epiphyseal scar, it is necessary to consider the manner of force transmission through the wrist and the potential effect that this may have on the persistence of the epiphyseal scar in the distal radius.

5.3.3 Discussion of the regional variation in the persistence of the epiphyseal scar in the distal radius

The results of previous analyses suggest that the potential obliteration of the epiphyseal scar observed in adult individuals may be influenced by factors other than those included within the remit of this study. Consequently, it is necessary to gain the maximum amount of information from the data relating to potential influences on the rate of bone remodelling within the distal radius which in turn will affect the persistence of the epiphyseal scar.

Analysis of the persistence of the epiphyseal scar in the medial, central and lateral regions of the distal radius did not reveal a statistically significant degree of variation in the regional persistence of the feature in any of the three regions in either females or males. The highest mean persistence score was observed in the central third of the bone in both sex cohorts. In addition, the central region of the bone was found to exhibit the lowest percentage of individuals in whom complete obliteration of the epiphyseal scar occurred and the highest percentages of individuals to whom TPS values of 3 or 4 were assigned in both sex cohorts. This indicates that a greater level of persistence of the epiphyseal scar may be

encountered within this region than within the medial or lateral regions of the distal radius. This suggests that the remodelling of the epiphyseal scar within this area is less than is encountered in either the medial or lateral region of the bone. Consequently, it is postulated that the central region of the distal radius is less exposed to those factors with the potential to influence the rate of remodelling of the epiphyseal scar than either the medial or lateral regions of the bone.

In contrast to the pattern observed in the region of the highest mean persistence rate of the epiphyseal scar between sex cohorts, the area with the lowest mean persistence score differed between females and males. Within the female cohort, the minimum lowest mean persistence rate was observed in the medial region of the distal radius. This region was also found to exhibit the highest percentage of individuals for whom persistence scores of 0 or 1 were assigned. This suggests that in female individuals, the remodelling rate within the medial third of the distal radius exceeds that observed in either the lateral or central regions. The medial region also exhibited the lowest percentage of individuals in whom a complete epiphyseal scar was observed. This indicates that bone remodelling within this area of the bone may occur at a faster rate than in other areas of the distal radius. It is reasonable therefore to suggest that in female individuals, this region is exposed to a greater degree of influence from extrinsic factors than either the central or lateral regions. Within the male sample, the lowest mean persistence score was found in the lateral third of the distal radius. This region was also found to exhibit the highest percentage of individuals for whom a persistence score of 0 was assigned and the lowest percentage of individuals for whom the remainder of the persistence scores were assigned. In addition, no male individuals were observed to retain a complete epiphyseal scar within this region of the bone. These results suggest that the rate of remodelling within the lateral third of the distal radius in male individuals exceeds that observed in either the central or medial regions of the bone and that the epiphyseal scar is likely to be retained to a lesser extent within this area of the distal radius than in the remainder of the bone.

Although suggestive of a pattern in the application of force, it was necessary to reinforce these findings with statistical analysis of the variation in persistence of the epiphyseal scar across the distal radius. Within both the female and male

samples, the results of a series of ANOVA tests found that there was a statistically significant degree of variation between the persistence scores assigned to the central third of the bone compared with those assigned to either the medial or lateral regions. There was no statistically significant difference in the persistence scores assigned to the lateral and medial thirds of the distal radius. These results suggest that although a difference in the mean persistence score of the medial and lateral regions was observed, the forces to which these areas are exposed may not result in a significant alteration to the rate at which the epiphyseal scar may be remodelled. As these regions were found to be statistically different from the central third of the bone, it is suggested that the factors which influence the remodelling of the epiphyseal scar are applied to a greater degree in the medial and lateral regions of the distal radius than in the central third of the bone irrespective of the sex of the individual.

Further analysis was undertaken to establish the statistical significance of the variation of persistence score between sexes within each region of the distal radius. The results of a series of one-way ANOVA suggested that there was no statistically significant difference in either the medial or central thirds of the distal radius. These results suggest that the drivers of remodelling of the epiphyseal scar within the medial and central region of the distal radius may be similar in both sexes, consequently, it is suggested that this may represent a functional force which is applied regardless of the sex of the individual. This pattern was not continued in the lateral third of the distal radius where a statistically significant degree of inter-sex variation in RPS values was observed, indicating that this region may be exposed to additional factors that may alter the rate of localised bone remodelling.

Before any hypotheses may be considered relating to the potential drivers of bone remodelling and the associated alteration to the epiphyseal scar in the distal radius, it was necessary to assess the relationship between chronological age, biological sex and side of the body on the persistence of the epiphyseal scar within discrete regions of the bone. The results of the GLM analyses suggest that there was a statistically significant relationship between chronological age and the persistence of the epiphyseal scar within the discrete regions of the distal radius.

This relationship, though significant, only explained 2.2% of the variation in the degree of persistence of the feature. In addition to chronological age, biological sex and region of the bone were found to exhibit statistically significant relationships with the persistence score. Of the interactions examined by these analyses, the strongest relationship with persistence score was observed with the combined effect of age, sex and side of the body. This model was found to explain 8.1% of the variation in persistence of the epiphyseal scar within the discrete regions of the distal radius. These results support the earlier findings which suggest that the variation in the epiphyseal scar may be due to factors other than those included in this study. It is therefore necessary to consider influences which may explain the variation in the persistence of the epiphyseal scar, including that which is attributable to the factors of age, sex and side of the body.

The proposed paradigm on which the following conclusions are based is that the degree of persistence of the epiphyseal scar is influenced by the mechanical loading to which the area of bone is exposed. Within the medial third of the bone, it is hypothesised that the applied force may be partially generated through the insertion of the interosseous membrane and the intra-articular pressures and forces associated with the distal radioulnar joint.

The role of the interosseous membrane in force transmission within the forearm has been a contentious issue within the literature, particularly in relation to its functional biomechanical role. It is suggested however that the intact interosseous membrane facilitates load transmission from the distal radius to the proximal ulna (Birkbeck *et al.*, 1997; McGinley and Kozin, 2001). In addition to its role in load transmission, the interosseous membrane also forms the attachment site for many muscles of the forearm (McGinley and Kozin, 2001). As a result, there may be an increase in the load applied to the bone through the action of the muscles which attach to the membrane, resulting in a concomitant increase in the tensile stresses to which the distal radius is exposed. While the insertion of the interosseous membrane places the medial aspect of the radius under tension, movement of the radius relative to the ulna may result in the shaft of the radius being placed under compression. This may therefore increase the rate of remodelling within the

medial aspect of the bone, thereby lessening the appearance of the epiphyseal scar in this region.

The distal radioulnar joint (DRUJ) comprises the articulation between the ulnar notch of the distal radius and the ulnar head and facilitates the movements of pronation and supination, through which the forearm and hand may traverse through 180° (Linscheid, 1992). In addition to permitting movement of the forearm, the DRUJ is also believed to play a role in load distribution within the forearm through bone-ligament interactions, where it has been suggested that the load passing through the ulna is similar to that which passes through the DRUJ (Shaaban *et al.*, 2004). Although no data have been located relating to the pressure exerted on the distal radius through its articulation with the distal ulna, it has been suggested that during pronation and supination, the volar and dorsal radioulnar ligaments respectively are placed under increased tension (Hagert, 1992; DiTano *et al.*, 2003). This may expose the medial aspect of the distal radius to intermittent mechanical stimuli that may have a positive effect on the rate of bone remodelling, potentially resulting in a localised increase in the rate of bone turnover and concomitant obliteration of the epiphyseal scar.

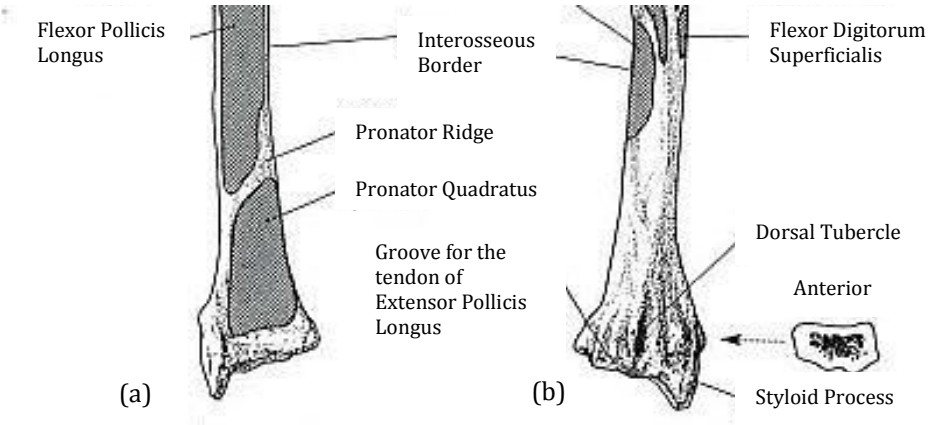

Figure 5.5: Muscular attachment sites of the distal radius in the (a) anterior and (b) posterior views. Adapted from Scheuer and Black (2000)

The central third of the distal radius, unlike the medial or lateral areas of the bone does not form the attachment site for a large number of powerful muscles, although it is inclusive of the attachment of the pronator quadratus muscle (Figure 5.5) (Standring, 2008). As a result, the load to which the central region of the distal radius is exposed may occur as a result of the axial load transmitted through the

radiocarpal joint. According to the literature, approximately 80% of the force applied to the wrist is transmitted by the radius, of which 60% is transmitted through the articulation of the scaphoid with the lateral articular facet of the radius, while the remaining 40% is transmitted via the articulation of the lunate with the medial articular facet of the distal radius. The distribution of force between the lunate and scaphoid articulations however is dependent on the position of the wrist in relation to the anatomical position (Palmer and Werner, 1984; Patterson and Viegas, 1995; Shaaban *et al.*, 2006; Majima *et al.*, 2008). It is presumed that the force applied to the distal radius through the radiocarpal joint will differ between sexes, according to the level of force generated through muscular contraction and applied external load. As the breadth of the distal radius is larger in males than females, the size of the articular surface will vary accordingly (Allen *et al.*, 1987; Sakaue, 2004; Barrier and L'Abbé, 2008). Consequently, the quantity of force applied per unit area may be equivalent in both sexes. This may explain the absence of a statistically significant difference in the persistence scores assigned to females and males in this region of the distal radius.

When tracked across the distal radius in a medial to lateral direction, the statistical significance of the inter-sex variation in assigned RPS values increased and reached its zenith in the lateral third of the bone. It is suggested that this area, being the only region of the bone in which statistically significant variation between females and males was found, is subjected to forces which vary significantly between the sexes, however they are similar to those applied to the medial third of the bone within each sex cohort. This may indicate that the force generated by the musculature of the forearm is applied to both the medial and lateral aspects of the radius. Within the medial region of the bone however, a portion of this force is transmitted by the interosseous membrane through its role as a load bearing structure (Birkbeck *et al.*, 1997; McGinley and Kozin, 2001). As no similar structure exists on the lateral aspect of the bone, this region must bear the full load and therefore may be more susceptible to loading related alteration to the appearance of the epiphyseal scar. As the functional requirements of the limb are consistent between the sexes, it is reasonable to hypothesise that the variation in the persistence of the epiphyseal scar may be influenced by the variation in

muscle mass found between females and males, particularly those which insert into the distal aspect of the radius such as brachioradialis (Janssen *et al.*, 2000; Doherty, 2001; Abe *et al.*, 2003). The contraction of muscles such as this may exert transient forces on the distal radius, resulting in increased levels of obliteration of the epiphyseal scar in the lateral third of the bone, as observed in this study.

The findings of this study challenge the traditional view of obliteration of the epiphyseal scar as a function of increasing age and indicate that the collective understanding of the temporal stability of this feature in the distal radius is incomplete. Consequently, the implications of the findings of this study on the interpretation of radiographic images of the distal radius for the purposes of skeletal age estimation are potentially significant.

6 Persistence of the epiphyseal scar in the distal femur

6.1 Sample Distribution

The distribution of the sample according to sex, age and side of the body from which the radiographs were obtained is presented in Table 6.1.

Table 6.1: Distribution of the sample used in the analyses of the distal femur according to chronological age, biological sex and side of the body

Age	Female Right	Female Left	Male Right	Male Left
20	5	5	5	5
21	3	3	5	5
22	3	5	5	4
23	4	4	5	5
24	5	5	5	4
25	4	5	5	5
26	5	4	5	5
27	5	4	5	5
28	3	4	3	5
29	4	4	5	5
30	5	4	5	3
31	4	5	4	4
32	5	4	5	5
33	5	5	5	4
34	5	5	4	5
35	5	4	5	4
36	4	4	5	5
37	5	5	5	4
38	4	5	4	5
39	3	5	5	4
40	3	5	5	4
41	4	4	5	2
42	5	5	5	5
43	5	5	5	5
44	4	5	5	3
45	5	4	5	5
46	5	4	5	5
47	5	5	5	4
48	5	4	5	5
49	4	5	5	5
50	4	2	5	5
Total	**135**	**137**	**150**	**139**

6.2 Results

6.2.1 Intra-Observer Analysis

Initially, a series of ANOVA were conducted to determine the variation in the assignment of TPS values by a single observer on two occasions for both the female and male sample. The results of these analyses suggested that the variation observed in the TPS assigned to female individuals on two occasions was not statistically significant (P=0.159); however a statistically significant difference was observed between the assigned TPS in the male sample (P=0.017).

Analysis of the data obtained from the intra-observer assessments showed that 80% and 70% agreement was observed in the female and male samples respectively.

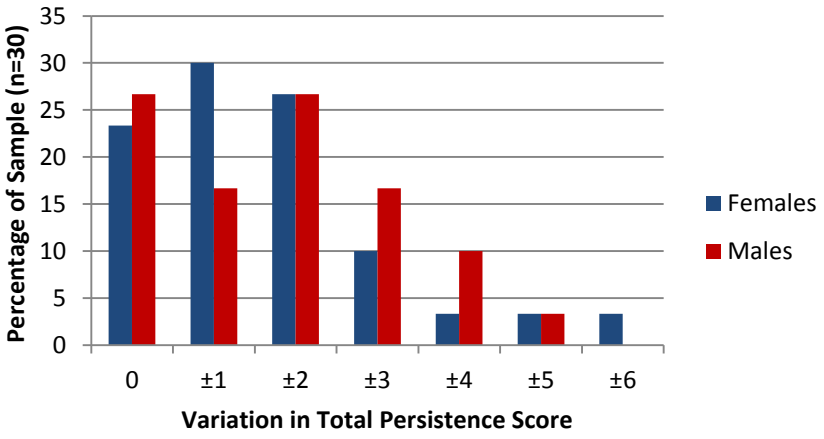

Figure 6.1: Intra-observer variation in Total Persistence Score assigned to the distal femur according to biological sex

The data presented in Figure 6.1 suggest that although the majority of scores are likely to fall within 2 of those assigned on a separate occasion, the maximum variation between scores assigned to the female sample was ±6. Within the male samples, the maximum variation between the TPS values assigned to a single individual was ±5. In both sex cohorts, the maximum variation in assigned TPS values was encountered at a frequency of 3.33%.

To assess the statistical relationship between the assigned TPS value and the round of assessment in which the analyses were made, a series of GLM analyses were conducted. The results of these analyses are presented in Table 6.2.

Table 6.2: Results of the General Linear Model analysis of the intra-observer variation in the distal femur

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	0.059	0.173	0.030	0.022	2.2%
RoA*	0.009	0.239	0.057	0.049	4.9%
Sex*RoA*	0.578	0.299	0.089	0.066	6.6%

*RoA = Round of Assessment

This analysis showed that there was a statistically significant relationship between round of assessment and TPS value assigned at the distal femur (P=0.009). The variation attributable to the round of assessment explained 4.9% of the total variation in assigned TPS value. When considered as co-variables, the relationship between sex and round of assessment and TPS was not found to be statistically significant (P=0.578).

6.2.2 Inter-Observer Analysis

Inter-observer consistency in the assignment of TPS was assessed to establish the repeatability of the scoring system presented in this study. Initially, a one-way ANOVA was undertaken to determine whether a statistically significant difference existed between the TPS assigned to females compared with males. To assess the degree of inter-observer consistency and the influence of experience on the repeatability of the method, the percentage agreement between each of the three observers was calculated and is presented in Table 6.3.

Table 6.3: Inter-observer percentage agreement in Total Persistence Score in the distal femur

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	83.33	70.00	90.00
Male	53.33	73.33	80.00

This analysis showed that the lowest percentage agreement in the female sample (70%) was found between the TPS values assigned by observers 1 and 3. The lowest percentage agreement within the male sample (53.33%) was observed between observers 1 and 2. The highest percentage agreement was consistently observed between observers 2 and 3 where agreement values of 90% and 80% were calculated for females and males respectively. Within the female sample, the greatest variation between two TPS values was ±5 and was found within the

interaction between observers 1 and 3 at a frequency of 6.67%. The greatest variation between two TPS values in the male sample was ±8 and was observed within the interaction between observers 2 and 3 at a frequency of 3.33%.

To provide a context in which the inter-observer percentage agreement may be interpreted, the statistical significance of the variation in the assignment of TPS between observers was calculated and the results presented in Table 6.4.

Table 6.4: Statistical significance of the inter-observer variation in the assignment of Total persistence Scores in the distal femur according to biological sex

Sex	Obs 1v Obs 2	Obs 1v Obs 3	Obs 2v Obs 3
Female	0.547	0.012	0.040
Male	0.135	0.006	0.162

These results show that the highest level of statistical significance in the variation between assigned TPS values occurred in the assessments of observers 1 and 3 in both the female and male samples. This indicates that the data derived from these analyses exhibited the greatest statistical variability. Of the remaining assessments, only that between observers 2 and 3 in the female sample showed a statistically significant level of variation between the TPS values assigned by each observer. As the only observer pairing in which no statistically significant variation was found, observers 1 and 2 were deemed to exhibit the greatest overall level of statistical consistency. These individuals represented the lowest and intermediate levels of experience in radiographic interpretation respectively.

To further examine the statistical relationship between observer and TPS, a series of GLM analyses were undertaken, the results of which are presented in Table 6.5.

Table 6.5: Results of the General Linear Model analysis of inter-observer variation in the distal femur

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	0.268	0.084	0.007	0.001	0.1
Observer	0.001	0.283	0.080	0.070	0.7
Sex*Observer	0.765	0.300	0.090	0.064	6.4

The results of this analysis show that when considered as the sole explanatory variable, observer exhibits a statistically significant relationship with TPS

(P=0.001); however variation in observer was found to explain only 0.7% of variation in TPS. From these results, it was also observed that when considered independently, the relationship between sex and TPS was not statistically significant (P=0.268). This was reinforced by the result of a one-way ANOVA which showed there to be no statistically significant variation between the TPS assigned to females and males (P=0.310). The combined relationship of sex and observer with TPS was not found to be statistically significant (P=0.765) in this anatomical region. The results of this study therefore suggest that when all explanatory variables are accounted for, there was no statistically significant variation in the assignment of TPS between observers. The method may therefore be considered repeatable.

6.2.3 Main Data Analysis

Initial analysis of the data showed that 99.26% of females and 97.23% of males were observed to exhibit some remnant of the epiphyseal scar at the distal femur, however the results of a one-way ANOVA showed that a statistically significant difference existed between the TPS assigned to females and males (F=48.269; P<0.001).

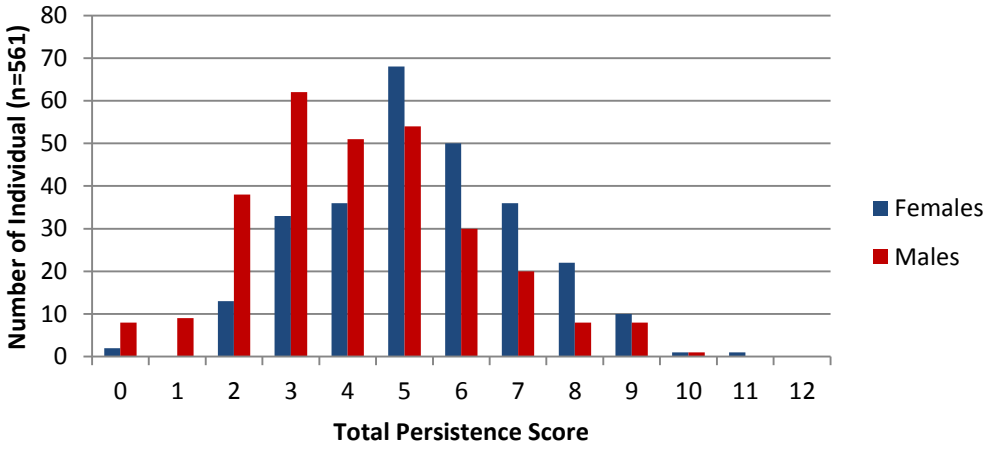

Figure 6.2: Distribution of the distal femur study sample according to biological sex and Total Persistence Score

The distribution of the sample according to TPS, presented in Figure 6.2, suggests that a similar pattern of distribution is observed in both the female and male subject groups. The results of Shapiro-Wilk normality tests showed that neither

the female (P<0.001; W-statistic= 0.971) or male (P<0.001; W-statistic=0.965) samples were distributed normally.

The mean chronological ages of individuals according to each persistence score are presented in Table 6.6 and Table 6.7.

Table 6.6: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal femur in female individuals

Total Persistence Score	Mean	Maximum (years)	Minimum (years)
0	33.50 (n=2)	47	20
1	--	--	--
2	31.85 (n=13)	43	20
3	31.21 (n=33)	40	20
4	33.06 (n=36)	49	21
5	35.59 (n=68)	50	20
6	37.66 (n=50)	48	20
7	36.39 (n=36)	50	20
8	37.27 (n=22)	50	23
9	34.50 (n=10)	48	21
10	41.00 (n=1)	41	41
11	26.00 (n=1)	26	26
12	--	--	--

Table 6.7: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal femur in male individuals

Total Persistence Score	Mean (years)	Maximum(years)	Minimum(years)
0	38.75 (n=8)	47	21
1	32.67 (n=9)	47	27
2	35.50 (n=38)	50	20
3	35.68 (n=62)	50	20
4	35.22 (n=51)	50	20
5	34.30 (n=54)	50	20
6	34.53 (n=30)	50	20
7	30.10 (n=20)	50	21
8	34.63 (n=8)	48	20
9	30.63 (n=8)	43	20
10	33.00 (n=1)	33	33
11	--	--	--
12	--	--	--

Within the female sample, no individuals were found to exhibit an epiphyseal scar of TPS 1 or TPS 12. In the male sample, no individuals were recorded as exhibiting an epiphyseal scar of TPS 11 or TPS 12. As TPS values represent a scale against which any trend in the mean chronological age of the individuals represented by

each persistence score may be measured, the net difference in mean chronological age between cohorts 2 and 7 was calculated for both sexes. These TPS values were selected as they represent the lowest and highest TPS cohorts, common to both sexes, in which n>10. These analyses showed that a positive net difference in the mean chronological ages assigned to TPS cohorts 2 and 7 occurred in both females (+4.84 years) and males (+5.4 years). This finding indicates that there may be a slight inverse relationship between mean chronological age and TPS value in the distal femur in both females and males.

The relationship between the percentage of individuals to whom a TPS value of 0 or TPS ≥9 was assigned and chronological age, linear regression analyses were conducted in the female and male sample data. The results of these analyses are presented in Figure 6.3 and Figure 6.4 for females and males respectively.

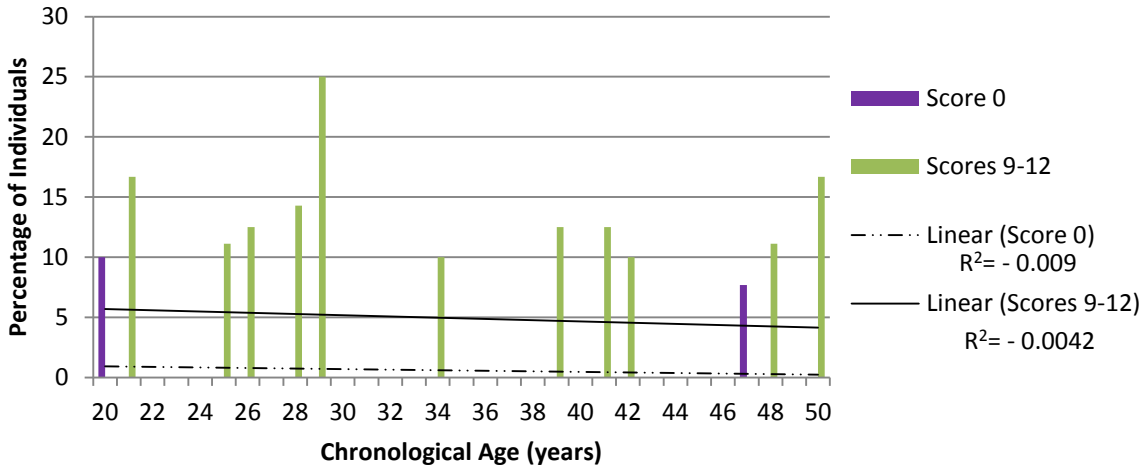

Figure 6.3: Percentage of female individuals exhibiting complete obliteration and maximum persistence of the epiphyseal scar in the distal femur according to chronological age

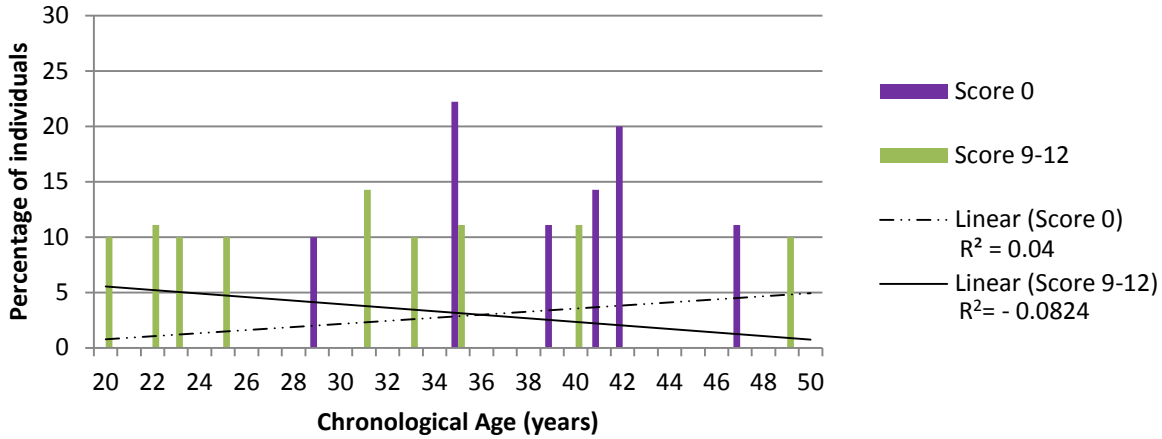

Figure 6.4: Percentage of male individuals exhibiting complete obliteration and maximum persistence of the epiphyseal scar in the distal femur according to chronological age

These analyses showed that in both the female and male samples, an inverse trend exists between the percentage of individuals to whom a TPS value ≥9 was assigned and increasing chronological age, although the strength of this relationship is stronger in males (R^2= -0.082) than females (R^2= -0.004). Within the female sample, a weak negative trend appears to exist between the percentage of individuals to whom a TPS value of 0 was assigned and increasing chronological age (R^2=-0.009).

The relationship between these factors in the male sample shows a stronger, positive trend (R^2=0.04), suggesting that the percentage of male individuals in whom complete obliteration of the epiphyseal scar was achieved was concomitant with increasing chronological age.

To assess the statistical relationship between chronological age, sex and side of the body with TPS, a GLM analysis was undertaken, the results of which are presented in Table 6.8.

Table 6.8: Results of the General Linear Model analyses in the distal femur

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.978	0.030	-0.025	0%
Sex	<0.001	0.080	0.078	7.8%
Side	0.663	0.000	-0.001	0%
Age*sex	0.058	0.181	0.081	8.1%
Age*side	0.276	0.093	-0.018	0%
Sex*side	<0.001	0.121	0.116	11.6%
Age*sex*side	<0.001	0.379	0.204	20.4%

These data suggest that when considered as the sole explanatory variable, the relationship between chronological age and TPS is not statistically significant (P=0.978). A statistically significant relationship was detected between sex and TPS (P<0.001); however the variation in sex explained only 7.8% of the variation within TPS. Although the relationship between total persistence score and side of the body was not statistically significant when considered independently (P=0.663), the interaction between sex and side was statistically significant (P<0.001) and explained 11.6% of variation in TPS. The influence of all three variables when considered as covarying factors on TPS was found to be statistically significant (P<0.001) and explained 20.4% of variation within TPS.

Given that both age and side were found to be insignificant, for the analysis of all three factors to yield a highly significant result, the effect of biological sex must be of sufficient significance to bias the analysis. To determine whether any bias was present within and between the sex and side-specific groupings a further series of one-way ANOVA were conducted. The results of these analyses are presented in Table 6.9.

Table 6.9: Reciprocal table of the statistical significance of variation in the assignment of Total Persistence Scores in the distal femur according to sex and side of the body

	Female Left	**Male Right**
Female Right	<0.001	<0.001
Male Left	0.195	<0.001

The results of these analyses showed that a significant difference was present between the TPS values assigned to images from the left and right sides of the body in both females and males. Further analysis suggested that there was no statistically significant difference between the left sided females and left sided males; however a statistically significant difference was found to exist between the data obtained from the right sided images of females and males. From these results, the data from the male right sided radiographs appear to be sufficiently different from other data groups to cause bias within the remainder of the sample.

The mean TPS value for each sex-specific and side-specific group was calculated to assess whether any similarities in persistence could be detected. These results are presented in Table 6.10.

Table 6.10: Mean total persistence score in the distal femur according to sex and side of the body

	Left	Right
Female	4.93	6
Male	4.62	3.79

These results showed that while the mean TPS values assigned to left distal femora in females and males were similar, there was an observable difference in the mean TPS values assigned to right distal femora in both sex groups.

To assess the variation in the persistence of the epiphyseal scar across the bone, the area was divided into anterior (tracks 1 and 2), central (tracks 3 and 4) and posterior (tracks 5 and 6) regions and the persistence score within each region was calculated. Initial analysis relating to the regional persistence of the epiphyseal scar was assessed through the calculation of the mean RPS value for each region. The resultant data are presented in Table 6.11.

Table 6.11: Mean regional persistence scores assigned to females and males in the distal femur

	Anterior	Central	Posterior
Female	1.91	2.37	1.05
Male	1.61	1.82	0.76

In addition to the calculation of the mean RPS values, the percentage of individuals for whom each score was assigned in each region was determined. The results of these analyses are presented in Table 6.12 and Table 6.13 for females and males respectively.

Table 6.12: Percentage distribution of Regional Persistence Scores in the distal femur in female individuals

Persistence Score	Anterior Region	Central Region	Posterior Region
0	3.68%	2.57%	28.31%
1	25.37%	9.56%	46.69%
2	48.53%	48.16%	16.91%
3	21.32%	27.94%	8.09%
4	1.10%	11.76%	0.00%
Total	**100%**	**100%**	**100%**

These results show that the mean persistence score assigned to the central region of the distal femur is greater than either the anterior or posterior regions in both the female and male samples. This is supported by the finding that the highest percentage of scores 3 or 4 were assigned to the central region in both the female and male samples.

Table 6.13: Percentage distribution of Regional Persistence Scores in the distal femur in male individuals

Persistence Score	Anterior Region	Central Region	Posterior Region
0	9.00%	9.69%	45.67%
1	38.06%	29.41%	37.37%
2	37.37%	35.29%	12.11%
3	13.84%	20.42%	4.84%
4	1.73%	5.19%	0.00%
Total	**100%**	**100%**	**100%**

These results suggest that the epiphyseal scar is less likely to be remodelled in the central region than in the anterior or posterior regions of the distal femur. The mean persistence scores assigned to the posterior region were lower than the anterior region in both females (1.05) and males (0.76) (Table 6.11). This is supported by the results obtained for the percentage of individuals in whom a score of 0 was assigned for the posterior region in both females (28.31%) (Table 6.12) and males (45.67%) (Table 6.13). These results suggest that the epiphyseal scar is more likely to be remodelled in the posterior region than in the anterior or central regions of the distal femur. It is also noted that while a greater percentage of individuals were assigned a score of 4 in the central region than either the anterior or posterior regions, there was a decline in this percentage relative to those to whom a score of 3 was assigned by 68% and 75% for females (Table 6.12) and males (Table 6.13Table 6.13 respectively. These results indicate that a degree of obliteration is likely to occur in at least 89.24% of females and at least 94.81% of males.

To assess the statistical relationship between chronological age, sex, side and region of the bone and persistence of the epiphyseal scar, a GLM analysis was undertaken. The results of this analysis are presented in Table 6.14.

Table 6.14: Results of the General Linear Model analyses for regional variation in the persistence of the epiphyseal scar in the distal radius

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.906	0.012	-0.006	0
Sex	<0.001	0.032	0.032	3.2
Side	0.630	0.000	0.000	0
Region	<0.001	0.229	0.228	22.8
Age*sex	0.011	0.073	0.038	3.8
Age*side	0.066	0.038	0.001	0.1
Sex*side	<0.001	0.049	0.047	4.7
Region*side	0.190	0.230	0.228	22.8
Region*sex	0.022	0.264	0.262	26.2
Region*age	0.948	0.261	0.218	21.8
Age*sex*side	<0.001	0.153	0.087	8.7
Region*side*sex	0.745	0.283	0.278	27.8
Region*side*age	0.677	0.313	0.228	22.8
Region*sex*age	0.913	0.345	0.264	26.4
Region*sex*age*side	0.974	0.468	0.318	31.8

These results suggest that when considered in isolation, a statistically significant relationship between region of the bone and persistence score was observed ($P<0.001$). This variation was found to explain 22.8% of the variation within the scores attributed to the three regions. This is supported by the results obtained from a series of one-way ANOVA, which are summarised in Table 6.15.

Table 6.15: Statistical significance of inter-region variation in regional persistence scores in the distal femur according to biological sex

	Anterior v Central	Central v Posterior	Anterior v Posterior
Female	<0.001	<0.001	<0.001
Male	0.013	<0.001	<0.001

These results suggest that statistically significant degrees of variation in the persistence of the epiphyseal scar exist between the anterior, central and posterior regions of the distal femur in both females and males and therefore supports the findings of the GLM analyses which indicate that the combined effects of region of the bone and sex of the individual significantly influences the persistence of the epiphyseal scar in this anatomical site.

The results of the remainder of the GLM analyses suggested that a statistically significant relationship existed between biological sex and the persistence of the epiphyseal scar within the three discrete regions of the bone ($P<0.001$; $R^2=0.032$).

This is supported by the results of a series of one-way ANOVA which determined that a statistically significant difference was present between the persistence of the epiphyseal scar in females and males within the anterior ($P<0.001$; $q=3.996$), central ($P<0.001$; $q=6098$) and posterior ($P<0.001$; $q=3.882$) regions. The relationship between biological sex and region and persistence score was also found to be statistically significant ($P=0.022$) and explained 26.2% of variation within the assigned persistence scores. Chronological age was not found to exhibit a statistically significant relationship with regional persistence of the epiphyseal scar when considered as the sole explanatory variable ($P=0.906$; $R^2= -0.006$).

6.3 Discussion of the persistence of the epiphyseal scar in the distal femur

6.3.1 Discussion of the intra-observer and inter-observer analysis in the distal femur

This study may represent the first examination of the persistence of epiphyseal scars in the distal femur. Consequently, it is imperative that the scoring system can be applied reliably and consistently by multiple observers; and that it can provide repeatable measures which remain consistent between observations. In response to this requirement, a series of assessments were carried out to determine the intra- and inter-observer consistency of the scoring system presented in this study when applied to the distal femur.

Within this study, intra-observer agreement was found to be higher in the female sample than the male sample, where 80% and 70% of second round assessments were within 2 TPS values of those initially assigned in females and males respectively. Although the overall percentage agreement was lower in the male sample, the range of variation within this data set was found to be smaller than in the female sample. As assessments of the male sample were undertaken after those of the female sample, these data indicate that while experience in the application of the scoring system may not confer a beneficial effect on the overall level of consistency, it may decrease the range of error between repeated observations. This finding is consistent with reports of the beneficial effect of training and practice may have on the precision and accuracy with which assessments of skeletal development are made (Cockshott and Park, 1983).

Further analysis of this data showed that while the intra-observer variation in TPS within the female sample was not statistically significant, this trend was not continued within the male sample where a statistically significant difference between the TPS values assigned on the first and second occasions was observed. The results of the GLM analysis conducted on the intra-observer data suggested that there was a statistically significant relationship between round of assessment and TPS when considered independently. Inclusion of biological sex as an explanatory factor however rendered the interaction between round of assessment and TPS not statistically significant. Taking into account the results of all analyses, this study suggests that although some variation may exist between TPS values assigned on multiple occasions, this discrepancy is not sufficient to render it statistically significant. The method may be considered reliable when applied to this region.

In addition to the assessment of intra-observer consistency, it was necessary to examine the reliability of assessments when made by multiple observers. This study found that in both sex cohorts, the highest percentage agreement occurred between observers 2 and 3. As these observers represented the highest levels of experience in radiographic interpretation and skeletal age estimation, this may indicate that some experience in these fields may be of benefit to the consistent application of the method by multiple observers. Although representing the highest level of inter-observer agreement, in the female sample, this interaction was found to exhibit a statistically significant degree of inter-observer variation. This result was not replicated in the male sample, where no statistically significant variation was observed in the inter-observer pairing with the greatest percentage agreement. The discrepancy between the results obtained for the female and male data sets may be related to the order in which assessments were conducted as analysis of the female sample was conducted prior to that of the male sample.

In contrast to this, the lowest level of inter-observer agreement occurred between observers 1 and 3 in the female sample; and 1 and 2 in the male sample. As observer 1 represented the lowest level of experience in the interpretation of radiographic images and skeletal age estimation, these findings support the potential role of experience in these fields in the repeatable application of the

method. As this study included only one individual without experience in these areas of skeletal assessment however, further specific analyses would be required to affirm the role of experience in inter-observer consistency in the assessment of the epiphyseal scar.

In both sex cohorts, the greatest degree of statistically significant variation was found in the interaction between observers 1 and 3. Although this pairing was found to exhibit the lowest percentage agreement in the female sample, the presence of statistically significant variation indicates that the ranges of errors between the two data sets were largest between these observers. This finding supports the suggested effect of experience on the consistency of application of the scoring system to the epiphyseal scar in the distal femur.

Further examination of the inter-observer data suggested that while a statistically significant relationship existed between observer and TPS when considered independently, once variation attributable to sex was taken into account, this relationship was not statistically significant. These data, when combined with the remainder of the results of the inter-observer analyses suggest that when viewed holistically, there is no statistically significant variation in the assignment of TPS by multiple observers.

6.3.2 Discussion of the overall persistence of the epiphyseal scar in the distal femur

As a constituent part of the knee joint, the distal femur has been included in a number of methods of age estimation which utilise multiple imaging modalities in addition to the examination of dry bone (Pyle and Hoerr, 1969; O'Connor *et al.*, 2008; Cameriere *et al.*, 2012; Dedouit *et al.*, 2012; Kausar and Varghese, 2012). Although these methods are based on the examination of skeletal maturation, the criteria employed and their interpretation may differ. It is a matter of contention within the literature whether the epiphyseal scar obliterates over time as a result of bone remodelling, or whether the structure may be retained to some degree in some anatomical areas and in adult individuals. The epiphyseal scar of the distal femur is included within the methods of age estimation developed by O'Connor *et al.* (2008; 2012) and Cameriere *et al.* (2012). While the potential persistence of the epiphyseal scar in adult individuals has been noted, the method presented by

Cameriere *et al*. (2012) applies a staging system in which the obliteration of the epiphyseal scar is considered as the final maturity criterion; thereby suggesting that the epiphyseal scar is not retained in adult individuals (O'Connor *et al.*, 2008; O'Connor *et al.*, 2012). As with other regions of the skeleton, the potential persistence of the epiphyseal scar in the distal femur in adult individuals has not been examined.

Through the analysis of data obtained from the assessment of the epiphyseal scar in the distal femur, 99.26% of females and 97.23% of males were observed to retain some remnant of the feature (i.e. TPS ≥1) when viewed in the medial-lateral plane. On further analysis of these data, it was found that while no subjects were assigned a TPS value of 12, a single female individual aged 26 years was assigned a TPS value of 11. These data indicate that the persistence of a complete epiphyseal scar in the distal femur is unlikely to occur in either sex cohort. At the opposite end of the spectrum, within the female sample, no subjects were assigned a TPS value of 1, but two individuals were found to exhibit maximum obliteration of the epiphyseal scar.

Further analysis of the data showed that a negative trend between the percentages of individuals in whom at least two-thirds (TPS≥9) of the epiphyseal scar was retained and increasing chronological age occurred in both the female and male samples. Although present in both sexes, the strength of this relationship was found to be greater in males than the females. The relationship between maximum obliteration (TPS 0) and chronological age was analysed in both sex cohorts using an equivalent process. These analyses showed that in the female sample, a weak negative trend in the percentage of individuals for whom maximum obliteration of the epiphyseal scar was observed with increasing chronological age. As a TPS value of 0 was only recorded in three individuals, these results should be interpreted with caution. In contrast, a mild positive trend was observed between the percentage of individuals for whom TPS 0 was assigned and increasing chronological age. In both the TPS 0 and TPS ≥9 cohorts, the relationship between the percentage of individuals represented by each cohort and increasing chronological age was stronger in males than females. These results therefore

suggest that chronological age may exert a stronger influence on the persistence of the epiphyseal scar in males than females.

In addition to indicating that variation in TPS value, and therefore the persistence of the epiphyseal scar, was unlikely to be due solely to chronological age, the findings of these initial analyses suggest that the persistence of the epiphyseal scar may vary between males and females. This finding was supported by the results obtained from a one-way ANOVA which determined that there was a statistically significant difference in the TPS assigned to females and males. As a result, the relationship between sex and TPS and the interaction between sex and age, and the combined influence of these factors on TPS were examined.

This study suggests that when assessed as the sole explanatory variable, a statistically significant relationship was found to exist between sex and TPS. In addition to the high level of statistical significance, this analysis suggested that 7.8% of variation in TPS was attributable to variation in biological sex. Neither chronological age nor side of the body was found to exhibit a statistically significant relationship with TPS. The relationship between biological sex and TPS, though weak, was of sufficient statistical significance to require further investigation, particularly in reference to the effect that side of the body may have on the persistence of the epiphyseal scar. The results of a GLM analysis determined that although when considered as the sole explanatory variable, side of the body did not exhibit a statistically significant relationship with TPS, the potential interaction of side of the body with sex, required further examination. The resulting analysis suggested that there was a statistically significant relationship between sex and side of the body and TPS when considered as covariables. This result indicates that although bilateral variation was not itself statistically significant in relation to the persistence of the epiphyseal scar, it may play a role in enhancing the effect of biological sex on TPS. This is supported by the increase in percentage variation explained by the joint model from 7.8% to 11.6%.

Due to the statistical significance of the relationship between sex and side in relation to TPS, further analyses were undertaken to determine the location of the

greatest variation between sex and side specific data sets. Initial analysis included the calculation of mean TPS values assigned to each sex and side specific cohort, the results of which showed that the greatest mean persistence in the epiphyseal scar was found in female right sided images. The lowest mean TPS value was found in male right sided femora. Using a series of one-way ANOVA, it was determined that a statistically significant variation existed between the TPS assigned to the left and right sides of the body in both females and males. A comparison of TPS obtained from left sided images subsequently showed that there was no significant difference between the TPS assigned to these images in females and males, however a significant difference was observed between the TPS values assigned to female and male right sided images. These data suggest that variation in TPS observed between sides of the body is predominantly due to variation in the persistence of the epiphyseal scar in the right limb. This discrepancy in obliteration of the epiphyseal scar between sides of the body may be due to a modification to the bone remodelling rate within the right limb compared with the left limb, perhaps as a result of variation in the quantity or strength of skeletal muscle in the dominant versus non-dominant limb (Hunter *et al.*, 2000). As a significant difference was found between the TPS values assigned to female and male right femora, it is reasonable to hypothesise that the extent to which the factor or factors responsible for the variation in remodelling rate affect the persistence of the epiphyseal scar between sides is, in part, dependent on the sex of the individual, perhaps as a result of hormonal status and systemic stimulation of bone remodelling (Compston, 2001).

As systemic influences on bone remodelling exert their effects throughout the skeleton, it is suggested that the variation in remodelling rate in the distal femur between sides of the body and sex of the individual may be due to extrinsic factors, specifically limb dominance and the associated increase in muscle mass. It is reported in the literature that the skeletal manifestations of limb dominance may arise from an increase in muscularity of the dominant limb over the non-dominant side due to the preferential functional loading of one side over the other (Ditroilo *et al.*, 2010; Blackburn, 2011). This is contested by the results of a study by Frontera *et al.* (1991) which found no statistically significant difference between

the skeletal muscle mass of the dominant and non-dominant limbs in their sample of males and females between 45 and 78 years of age. A study by Hunter *et al*. (2000) however found that the maximal voluntary contraction of the knee extensor muscle group as a measure of muscle strength was significantly greater in the dominant limb when compared to the non-dominant limb in a sample of healthy females between 20 and 69 years of age. As this study included individuals of younger chronological age than were included in the study sample of Frontera *et al.* (1991) it is suggested that within younger cohorts, limb dominance exerts a greater influence on muscle mass than occurs within older cohorts due to age related muscle loss and the progressive masculinisation of the skeletal system in postmenopausal women (Doherty, 2001).

This study found that females exhibited a higher persistence rate than males and a greater mean persistence score than males in both the left and right cohorts. This suggests that a greater degree of remodelling has taken place within the distal femora of males than females, and within right femora than left in both sexes. As males generally have a higher proportion of skeletal muscle than females, the potential influence of dominance related muscle mass on remodelling rate is greater in males than females (Frontera *et al.*, 1991; Doherty, 2001; Abe *et al.*, 2003; Ditroilo *et al.*, 2010). The results obtained by this study suggest that the increased muscle mass in the dominant limb, regardless of sex, may cause an increased rate of osseous remodelling and may therefore explain the observed increase in the obliteration of the epiphyseal scar in this cohort.

Evidence within the literature suggests that although a difference in muscularity is present between sexes, the quantity of skeletal muscle possessed by an individual shows a gradual decrease with increasing chronological age (Lindle *et al.*, 1997; Kyle *et al.*, 2001; Doherty, 2003; Lee *et al.*, 2007). This age related loss of muscle, or sarcopenia, has been found to be more extensive in males than females, possibly as a function of a greater initial muscle mass (Lindle *et al.*, 1997; Doherty, 2001; Kyle *et al.*, 2001). As this study found no statistically significant relationship between TPS and chronological age, it does not appear that sarcopenia exerts a strong influence on the obliteration of the epiphyseal scar (Doherty, 2001). This

could be due to the relatively low degree of muscle loss expected within the age ranges included in this study (Doherty, 2001).

Although chronological age was not found to exhibit a statistically significant interaction with TPS when considered independently or in combination with an additional factor, this study suggests that the combined influence of the triumvirate of chronological age, biological sex and side of the body exerts a statistically significant effect on the persistence of the epiphyseal scar in the distal femur. This model was also found to account for the largest percentage of variation in the assignment of TPS values. Due to the absence of a statistically significant interaction between TPS and age or side of the body when considered independently, these data indicate that the statistical significance of the interaction between biological sex and TPS is of sufficient strength to mask the effects of the inclusion of non-significant variables.

This study suggests that although the combined influence of chronological age, biological sex and side of the body explains 20.4% of the variation in the persistence of the epiphyseal scar, the mechanism through which this occurs has yet to be explored. To examine the data further with a view to elucidating the mechanism through which this variation occurs, the persistence of the epiphyseal scar within three discrete regions of the distal femur was undertaken.

6.3.3 Discussion of the regional variation in the persistence of the epiphyseal scar within the distal femur

To facilitate a more detailed discussion of the potential influences on the persistence of the epiphyseal scar in the distal femur, the variation in the persistence of the feature across the growth plate in the anterior-posterior plane was assessed through the calculation of RPS values. Initial analyses found that a higher mean persistence score was assigned to the central region of the bone in both females and males while the lowest mean persistence scores were observed in the posterior region in females and males.

A statistically significant difference was found between the persistence scores assigned to three discrete regions of the growth plate in both females and males. Further analyses showed that the variation between all pairwise combinations in

the female sample were statistically significant. Within the male sample it was found that while the variation between the anterior and posterior, and central and posterior regions were statistically significant, the variation between anterior and central regions was not statistically significant. These results suggest that in male individuals, bone turnover in the posterior third of the distal femur may occur at a faster rate than in the anterior or central thirds of the bone. Consequently, the epiphyseal scar may be more likely to undergo a greater degree of remodelling in this region than in the remainder of the bone. This hypothesis is supported by the results of subsequent analyses which showed that the greatest percentage of individuals to whom a persistence score of 4 was assigned occurred in the central region for both females and males.

Within the anterior region, the percentage of individuals for whom a persistence score of 4 was assigned had decreased in both sex cohorts. Within the posterior region, no individuals were observed to retain a complete epiphyseal scar; however 8.09% and 4.84% of females and males respectively were assigned a persistence score of 3 within the posterior third of the bone. These results suggest that partial obliteration of the epiphyseal scar may occur in at least 89% of the female population and 94% of the male population.

The highest percentage of individuals for whom a score of 0 was assigned occurred in the posterior region. In contrast, the smallest percentage of individuals for whom this score was assigned was observed in the central region. These results therefore represent the inverse of those observed in the analysis of the assignment of a persistence score of 4. From this analysis it is suggested that the epiphyseal scar is likely to persist to the greatest extent in the central region and is most likely to undergo complete obliteration within the posterior region of the distal femur in both females and males. Although it is apparent that osseous remodelling occurs throughout the area in which the epiphyseal scar is situated, it is suggested that bone turnover may occur at a faster rate in the posterior third of the bone than encountered in the central third of the bone.

Through this analysis it was determined that when considered in isolation, there was a statistically significant relationship between region of the bone and TPS. In

addition, this relationship was found to explain 22.8% of the variation in TPS. The explanatory power of this relationship was enhanced by the inclusion of variation attributable to the sex of the individual. Although the statistical significance of this relationship declined, the explanatory power of the interaction between biological sex and region of the bone increased to account for 26.2% of variation in the assigned persistence scores. These findings were supported by the results of subsequent analyses which showed that a statistically significant difference was present between the persistence scores assigned to females and males in each region of the bone. These results further expose the underlying variation in persistence of the epiphyseal scar between females and males in the distal femur.

This study indicates that there is a statistically significant variation in the persistence of the epiphyseal scar across the distal femur in an anterior to posterior direction. A small degree of variation could be attributed to the positioning of the radiographic image and the degree to which the posterior border of the femoral condyle extended beyond the epiphyseal scar; however it is hypothesised that the variation in the persistence of the epiphyseal scar in the distal femur occurs as a result of an increase in the rate of remodelling in the posterior third of the bone relative to the anterior and central regions. As the rate of remodelling is influenced by both extrinsic and intrinsic factors, it is necessary to consider the variation in the effect of these factors on different regions of the bone and the potential impact this may have on the variability in the obliteration of the epiphyseal scar in the distal femur.

The results of this study suggest that there was no statistically significant relationship between age or side of the body and regional persistence of the epiphyseal scar. These results suggest that those factors which relate directly to the age of the individual or the side of the body on which the assessments were made do not exert a statistically significant influence on the preservation of the epiphyseal scar. When considered in the context of the region of the bone or the sex of the individual, this study has found that the inclusion of variation attributable to age enhances the variation attributable to sex.

There are several potential explanations for the variation in persistence of the epiphyseal scar between the anterior, central and posterior regions of the distal femur, including the effect of the surrounding musculature and the transmission of force through the distal femur. Conventional theory suggests that an alteration to the level of strain to which a bone, or part thereof, is exposed will affect the rate of localised bone remodelling to ensure that the structural competency of the bone is maintained (Frost, 1987; 1998b; Huiskes *et al.*, 2000). Using this approach as the basis of a hypothesis, the results of this study suggest that there may be a differential distribution of force across the bone with the minimum load being applied to the central third and the maximum load being transmitted through the posterior third. The degree of loading applied to the femur is dependent largely on the body mass of the individual with between 200-400% of the individual's weight being transmitted through the joint during level walking (Kutzner *et al.*, 2010). As males generally exhibit a larger total body mass than females, the force to which the distal femur is exposed is increased relative to their female counterparts (Kyle *et al.*, 2001). It is therefore reasonable to hypothesise that male individuals may undergo osseous remodelling at a faster rate and consequently, an increased level of obliteration of the epiphyseal scar may be expected in males relative to females. This hypothesis is supported by the results of this study which found that males exhibited a lower persistence of the epiphyseal scar than females in all three regions of the distal femur. The results of this study suggest that the greatest obliteration of the epiphyseal scar occurs in the posterior third of the distal femur. Consequently, it is reasonable to hypothesise that the anterior and posterior thirds of the distal femur are exposed to forces which require the bone within these regions to remodel at a faster rate than the central region of the bone and as a result, exhibit lower levels of persistence of the epiphyseal scar than the central third of the distal femur.

Due to its dual function as a structural support and locomotive apparatus, the femur is exposed to loading from multiple sources (Aiello and Dean, 1990). Although it has been suggested that calculation of specific stress trajectories within this region is problematic, a degree of trabecular organisation has been observed

which corresponds to the assumed force trajectories (Gaynor Evans, 1965; Palastanga and Soames, 2012). The complex nature of the forces to which the femur is exposed may result in variable rates of osseous remodelling within a single element. To attempt to understand the effect of applied load and bone turnover on the persistence of the epiphyseal scar, it is necessary to consider the intrinsic structure of the bone and the interactions between hard and soft tissues and the rate of remodelling within the cancellous structure of the distal femur.

The cancellous structure of the distal femur is aligned along the principal axes of compression and tension (Smith, 1962). Under axial loading, such as occurs during normal standing, the femur may be expected to undergo bending, placing the anterior-lateral surface of the bone under tension and the posterior-medial surface under compression (Gaynor Evans, 1965; Taylor *et al.*, 1996). Although bone may be less likely to fail under compression than under tension, the area of the bone under compressive loading is likely to undergo remodelling at a faster rate than that which is not exposed to such loading forces (Gaynor Evans, 1965). Consequently, it is suggested that the anterior and posterior thirds of the distal femur may be exposed to greater loads than the central third of the distal epiphysis. As a result, remodelling of the cancellous bone within these regions may occur at a faster rate than within the central region of the bone which is not under loading from bending or shear (Gaynor Evans, 1965). This is indicative of a variation in the degree to which the epiphyseal scar is remodelled within the distal femur and could suggest that the rate at which this turnover occurs is dependent on the load to which the bone is exposed.

In addition to the load applied to the femur by normal body weight, the anterior and posterior aspects of the distal femur form attachment sites for muscles of the thigh and the posterior compartment of the leg. The combination of these muscles, particularly the vastus muscle group and gastrocnemius muscle in the anterior compartment of the thigh and posterior compartment of the leg respectively influence the internal axial loading of the distal third of the femur (Duda *et al.*, 1997). The increase in loading by tensile forces caused by muscular contraction may result in a greater requirement for osseous remodelling, which in turn, may

stimulate further bone remodelling and subsequent obliteration of the epiphyseal scar in these regions, as suggested by the results of this study.

In addition to considering the potential effects of mechanical loading on the level of obliteration or persistence of the epiphyseal scar, it is also necessary to acknowledge the potential effect of radiographic superimposition on the interpretation of the epiphyseal scar in the distal femur. As radiographic images constitute a 2 dimensional representation of a 3 dimensional structure, variation in the quantity of bone (i.e. cortical or trabecular thickness, density etc.) may affect the observation and interpretation of the epiphyseal scar. As analysis of the distal femur was undertaken on radiographs in the M-L plane, an alternative explanation for the relatively greater persistence of the epiphyseal scar within the central third of the bone may be partially attributable to the location of the thickest area of bone. Conversely, the gross morphology of the anterior and posterior thirds of the bone may result in a lesser quantity of bone through which the x-rays must be transmitted, thereby resulting in a comparatively weaker radio-opaque line.

Although the posterior third of the distal femur appears to exhibit the lowest degree of persistence of the epiphyseal scar, this may be as a result of the orientation in which the distal femur was examined. As this study utilised clinical radiographic images, the positioning of the limb within the radiograph could not be controlled and consequently the angle at which the images were taken may have varied. These inconsistencies between images may therefore have resulted in variation in the degree of superimposition encountered in the posterior aspect of the femoral condyles and therefore introduced greater uncertainty in the observation and examination of the radio-opaque line of increased relative density which represents the epiphyseal scar.

7 Persistence of the Epiphyseal Scar in the Proximal Tibia

7.1 Sample distribution

The sample distribution according to age, sex and side of the body are presented in Table 7.1.

Table 7.1: Distribution of the sample used in the analysis of the proximal tibia according to chronological age, biological sex and side of the body

Age (Years)	Female Left	Female Right	Male Left	Male Right
20	5	5	5	5
21	5	5	5	5
22	5	5	5	5
23	5	5	5	5
24	5	5	5	5
25	5	5	5	5
26	5	5	5	5
27	5	5	5	5
28	5	5	5	5
29	5	5	5	5
30	5	5	5	5
31	5	5	5	5
32	5	5	5	5
33	5	5	5	5
34	5	5	5	5
35	5	5	5	5
36	5	4	5	5
37	5	4	5	5
38	5	5	5	5
39	5	5	5	5
40	5	5	5	5
41	5	5	4	5
42	5	5	5	5
43	5	5	5	5
44	5	5	5	5
45	5	5	5	5
46	5	5	5	5
47	5	5	5	5
48	5	5	5	5
49	5	5	5	5
50	5	5	5	5
Total	**155**	**153**	**154**	**155**

7.2 Results

7.2.1 Intra-observer analysis

Initially, a series of one-way ANOVA were conducted to determine the consistency of the assignment of TPS in repeated assessments made by a single observer. These analyses suggested that there was no significant difference between the TPS assigned during first and second rounds of assessment in either the female (P=0.115) or male (P=0.260) samples. The statistical power of the analyses did not reach the threshold of 0.8. A further analysis was undertaken to determine whether a statistical difference existed between the TPS assigned to females and males included in the intra-observer analysis. Although the results of this test suggested that there was no statistical difference between the groups (P=0.599), the threshold of statistical power was not reached.

From the analysis of the intra-observer data, percentage agreements of 80% and 83.33% were obtained for the female and male samples respectively as defined by the terms presented in section 3.4.1.

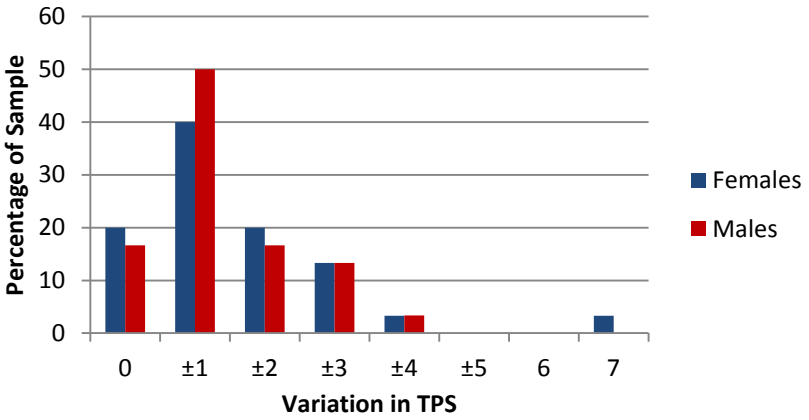

Figure 7.1: Distribution of the proximal tibia study sample according to biological sex and Total Persistence Score

As shown in Figure 7.1, there is a similar pattern in the variation observed within the intra-observer analysis in both females and males. With the exception of a single female individual, the scores assigned on first and second occasions did not differ by more than four scores.

To assess the statistical relationship between TPS assigned during the first and second rounds of assessment, a GLM analysis was undertaken, the results of which are presented in Table 7.2.

Table 7.2: Results of the General Linear Model analysis of intra-observer variation in the proximal tibia

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	0.599	0.044	0.002	-0.006	0
Round	0.052	0.179	0.032	0.023	2.3
Sex*Round	0.658	0.190	0.036	0.011	1.1

This analysis showed that there was no significant difference between TPS scores assigned at first and second rounds of assessment when considered as either a single factor (P=0.052) or as a covarying factor when considered with sex (P=0.658). In addition, the result of a one-way ANOVA suggested that there was no significant difference between the TPS assigned to females and males (P=0.599), however this analysis did not reach the threshold of statistical power (0.8). The analysis suggests that any variation which exists between TPS assigned by the same observer is not statistically significant and therefore suggest that this method is consistent when applied by a single observer.

7.2.2 Inter-observer analysis

Initially, a one-way ANOVA was undertaken to determine the significance of any variation which existed between the TPS assigned to sex specific groups. The results of this analysis suggested that the variation between females and males was statistically significant (P=0.027; H=4.890). Following this result all subsequent analyses of variance were undertaken in sex-specific groups. These results suggested that the variation in TPS assigned to the subsample by multiple observers was not statistically significant in either the female (P=0.730; H=0.630) or male (P=0.266; H=2.646) samples. Although variation between assigned TPS values was not found to be statistically significant, it was prudent to determine the percentage agreement between observers, the results of which are presented in Table 7.3.

Table 7.3: Inter-observer percentage agreement in Total Persistence Score in the proximal tibia

Sex	Obs 1 v Obs 2	Obs 1 v Obs 3	Obs 2 v Obs 3
Female	86.67	86.67	100.00
Male	66.67	80.00	83.33

This analysis found that the lowest percentage agreement was observed between observers 1 and 2 in both the female and male samples, although within the female sample, the value obtained for the percentage agreement between observers 1 and 2 equalled that obtained from the comparison of observers 1 and 3. The highest percentage agreement in the female and male samples was observed between the TPS values assigned by observers 2 and 3. The maximum variation in TPS values within the female sample was ±8 scores, which occurred in 3.33% of comparisons within the interaction between observers 1 and 2. Within the male sample, the greatest variation in TPS values was ±6 scores. This was detected in the interactions between observers 1 and 2 and 1 and 3, however this discrepancy was recorded at a greater frequency in the interaction between observers 1 and 3 (6.66%) than observers 1 and 2 (3.33%). A series of one-way ANOVA were conducted to assess the statistical significance of the variation in assigned TPS values between observers. These analyses showed that no statistically significant variation existed between any of the observers in either sex cohort (Table 7.4).

Table 7.4: Statistical significance of inter-observer variation in the assignment of Total Persistence Scores in the proximal tibia according to biological sex

Sex	Obs 1 v Obs 2	Obs 1 v Obs 3	Obs 2 v Obs 3
Female	0.988	0.626	0.633*
Male	0.123	0.311*	0.641

* Statistical power <0.8

Although the variation between observers was not found to be statistically significant in either sex group, it was deemed appropriate to quantify the relationship between observer and TPS. This was achieved through the application of a GLM analysis, the results of which are presented in Table 7.5.

Table 7.5: Results of the General Linear Model analysis of inter-observer variation in the proximal tibia

Factor(s)	P-Value	R	R^2	Adjusted R^2	% variation explained
Sex	0.036	0.158	0.025	0.019	1.9
Observer	0.221	0.130	0.017	0.006	0.6
Sex*Observer	0.618	0.217	0.047	0.019	1.9

These results showed that the variation in TPS which could be attributed to variation in observer represented 0.6% of the total variation in the sample. Variation in sex combined with that of observer accounted for 1.9% of variation in TPS; however this relationship was not found to be statistically significant (P=0.618).

The results of the inter-observer analyses suggest that the method may be consistently applied by multiple observers at the proximal tibia without significant variation in the overall assignment of TPS.

7.2.3 Main data analysis

Initial observation and analysis of the data presented in Figure 7.2 suggested that there was a similar distribution of TPS in both female and male sample groups.

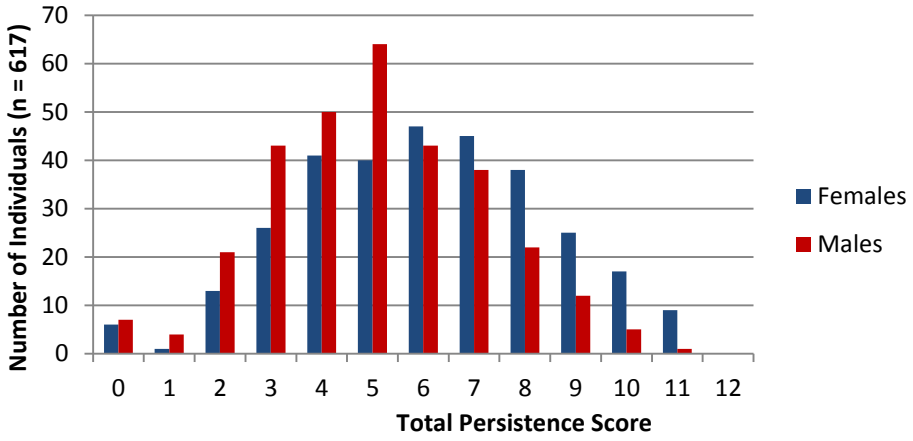

Figure 7.2: Distribution of the proximal tibia study sample according to biological sex and Total Persistence Score

This was supported by the results of a Shapiro-Wilk test which determined that although neither sample was normally distributed, the distributions of the female and male sample were statistically equivalent (W-statistic=0.977; P<0.001). The

results of a one-way ANOVA showed that there was a statistically significant difference in the TPS assigned to females and males ($P<0.001$).

Within the female subject group, although 98.05% of individuals were observed to exhibit some remnant of an epiphyseal scar, only 58.77% were found to retain an epiphyseal scar to which a TPS value of ≥6 was assigned. The total persistence rate within the male subject groups was marginally lower than that observed in the female sample. Total persistence rate within the male sample was recorded as 97.74% and only 39.03% of individuals were observed to retain an epiphyseal scar to which a TPS value of ≥6 was assigned.

The mean, maximum and minimum chronological ages of individuals according to TPS are presented in Table 7.6 and Table 7.7 for females and males respectively.

Table 7.6: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the proximal tibia in female individuals

Score	Mean (years)	Maximum (years)	Minimum (years)
0	41.17 (n=6)	47	32
1	39.00 (n=1)	39	39
2	43.38 (n=13)	50	21
3	34.58 (n=26)	49	20
4	39.66 (n=41)	50	22
5	35.95 (n=40)	50	20
6	34.57 (n=47)	50	20
7	33.51 (n=45)	50	20
8	33.32 (n=38)	50	20
9	31.84 (n=25)	50	20
10	29.94 (n=17)	42	20
11	28.56 (n=9)	41	20
12	--	--	--

As the TPS values represent a scale against which the mean chronological age of individuals represented by each cohort may be measured, the net difference in the mean chronological ages assigned to TPS values 2 and 9 were calculated for both sexes. These TPS values were selected as they represent the lowest and highest TPS values, where $n>10$, common to females and males. These analyses showed that a decrease in mean chronological age of 1.84 years was observed in males while in females, the difference in mean chronological ages between these cohorts was 11.54 years. These findings therefore suggest that an inverse trend may exist

between mean chronological age and increasing TPS value in both the female and male samples.

Table 7.7: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the proximal tibia in male individuals

Score	Mean	Maximum (years)	Minimum (years)
0	34.71 (n=7)	44	20
1	32.50 (n=4)	45	25
2	31.81 (n=21)	49	20
3	33.88 (n=43)	50	21
4	36.60 (n=50)	50	20
5	36.81 (n=64)	50	20
6	35.47 (n=43)	50	20
7	36.45 (n=38)	50	21
8	32.41 (n=22)	50	20
9	30.33 (n=12)	45	20
10	30.20 (n=5)	47	20
11	28.00 (n=1)	28	28
12	--	--	--

Further analysis of the data was undertaken to assess the relationship between maximal persistence and maximum obliteration of the epiphyseal scar with chronological age. The results of these analyses including lines of simple linear regression and are presented in Figure 7.3 and Figure 7.4 for females and males respectively.

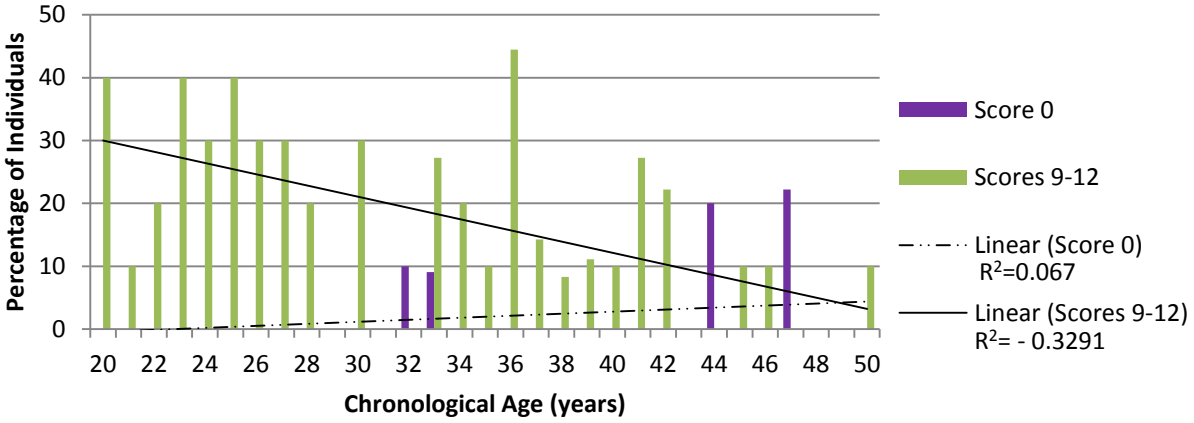

Figure 7.3: Percentage of female individuals exhibiting complete obliteration or maximum persistence of the epiphyseal scar in the proximal tibia

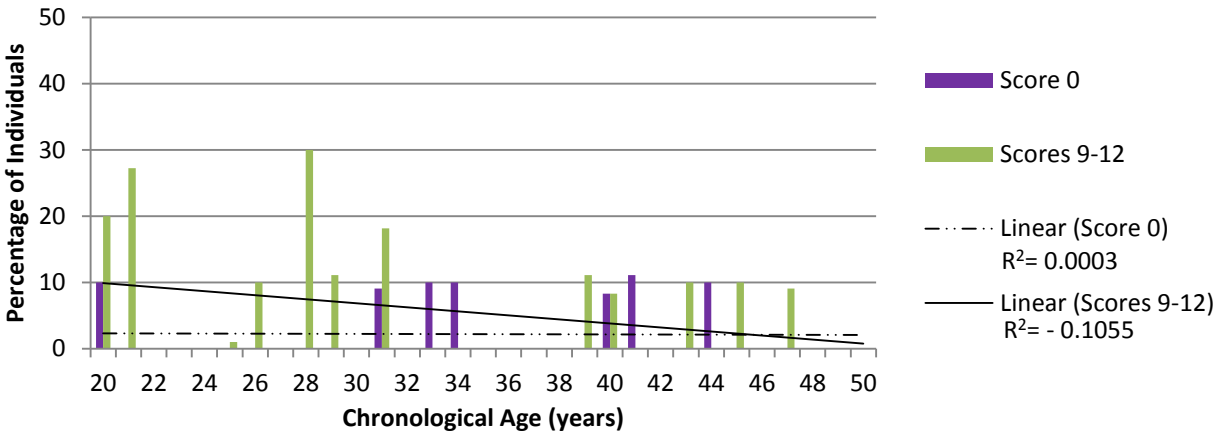

Figure 7.4: Percentage of male individuals exhibiting complete obliteration or maximum persistence of the epiphyseal scar in the proximal tibia

The results of these analyses showed that a negative trend exists between the percentage of individuals to whom a TPS value of ≥9 was assigned and increasing chronological age in both the male and female sample. Addition of lines of linear regression showed that this relationship was stronger in the female sample (R^2=0.329) than the male sample (R^2=0.105). These results also suggested that females were more likely to be assigned a TPS value ≥9 and therefore more likely to retain a higher proportion of the epiphyseal scar at the proximal tibia.

Within the cohort of female individuals for whom a TPS value of 0 was recorded, a positive trend was present between TPS and chronological age (R^2=0.067). In contrast, within the male sample, the trend in the percentage of individuals represented by TPS 0 was observed to remain constant (R^2=0.0003) at a rate of approximately 2.5% throughout the sample population.

The low degree of observed variation between the age cohorts suggests that other factors, in addition to age, may exert an influence on the persistence of the epiphyseal scar. To determine the influence of intrinsic factors, the data were analysed using a GLM. This facilitated the comparison of factors when considered as independent and codependent factors. The results of the GLM are presented in Table 7.8.

Table 7.8: Results of the General Linear Model analyses in the proximal tibia

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	0.029	0.074	0.027	2.7%
Sex	<0.001	0.047	0.045	4.5%
Side	<0.001	0.037	0.036	3.6%
Age*sex	0.03	0.19	0.101	10.1%
Age*side	0.555	0.156	0.063	6.3%
Sex*side	0.532	0.084	0.08	8%
Age*sex*side	0.384	0.313	0.147	14.7%

These data suggest that there is a significant relationship between chronological age and persistence of the epiphyseal scar at the proximal tibia (P=0.029). This model only explains 2.7% of the total variation in TPS. Both biological sex (P<0.001) and side of the body (P<0.001) displayed a statistically significant relationship with TPS. Although the interactions between these factors and TPS were of greater significance than that of chronological age, the percentage variation explained by the models is small, with 4.5% and 3.6% of variation being explained by sex and side respectively. Only the interaction between biological sex and chronological age displayed a statistically significant relationship with TPS (P=0.03). This association only accounted for 10.1% of variation observed within the TPS data.

As the results of the GLM analyses suggested that there was a statistically significant relationship between side of the body and TPS, further examination of this data was undertaken, through a series of one-way ANOVA, to assess the variation between sides of the body within and between sex cohorts.

Table 7.9: Reciprocal table of the statistical significance of variation in the assignment of total persistence score in the proximal tibia according to sex and side of the body

	Female Left	Male Right
Female Right	<0.001	<0.001
Male Left	<0.001	<0.001

The results of these analyses are presented in Table 7.9 and show that a statistically significant degree of variation in the persistence of the epiphyseal scar between left and right sides of the body was present within and between sex cohorts. Further analysis showed that in both females and males, the highest mean TPS value occurred in the left sided cohort. The mean TPS values for all cohorts

when categorised according to sex and side of the body are presented in Table 7.10.

Table 7.10: Mean total persistence score in the proximal tibia according to biological sex and side of the body

	Left	Right
Female	6.555	5.542
Male	5.474	4.652

This analysis also showed that in both the left and right sides of the body, a higher mean TPS value occurred in the female sample than in the male sample. These results, together with those presented in Table 7.9, are suggestive of localised factors which may alter the rate at which bone remodelling occurs between limbs both within and between sexes. To assess the regional specificity of these influences, analysis of the persistence of the epiphyseal scar within three discrete regions of the proximal tibia was undertaken.

Initial analyses showed that in both sex cohorts, the highest mean regional persistence score (RPS) value occurred in the medial third of the proximal tibia. Similarly, the lowest mean RPS value was observed in the lateral third of the bone (Table 7.11).

Table 7.11: Mean regional persistence scores in the proximal tibia according to biological sex

	Medial Region	Central Region	Lateral Region
Female	2.37	2.05	1.64
Male	2.02	1.63	1.41

These results also show that in all regions, a higher mean percentage score was found in females than males. This suggests that female individuals may be exposed to lower levels of influence on the remodelling of the epiphyseal scar than males across the expanse of the proximal tibial growth plate.

Further analysis was undertaken to assess the distribution of RPS values among individuals in the medial, central and lateral thirds of the proximal tibia in both sex cohorts. The results of these analyses are presented in Table 7.12 and Table 7.13 for females and males respectively.

Table 7.12: Percentage distribution of Regional Persistence Scores in the proximal tibia in female individuals

n=308	Medial	Central	Lateral
0	5.84	9.74	23.38
1	8.77	25.00	17.53
2	34.42	28.25	36.36
3	44.48	25.00	17.53
4	6.49	12.01	5.19
Total	**100%**	**100%**	**100%**

Table 7.13: Percentage distribution of Regional Persistence Scores in the proximal tibia in male individuals

n=309	Medial	Central	Lateral
0	7.77	15.53	23.62
1	12.94	31.39	29.13
2	50.81	32.36	31.72
3	26.21	16.18	13.59
4	2.27	4.53	1.94
Total	**100%**	**100%**	**100%**

These analyses showed that in both sex cohorts, the highest percentage of individuals in whom maximum obliteration of the epiphyseal scar was observed occurred in the lateral third of the proximal tibia. The medial third of the bone was also observed to exhibit the lowest percentage of individuals in whom maximum obliteration of the epiphyseal scar occurred in both females and males. Equivalent distributions of individuals to whom RPS values of 3 or 4 were assigned were also observed in females and males. For both sex cohorts, the highest percentage of individuals for whom an RPS value of 3 was assigned was observed in the medial third. The highest percentage of individuals who exhibited complete regional persistence of the epiphyseal scar occurred in the central third of the proximal tibia in both sexes. The lowest percentage of individuals to whom these RPS values were assigned was observed in the lateral third in both females and males.

From the analyses presented in Table 7.12 and Table 7.13, it was also observed that, with the exception of RPS 2 in the lateral third, RPS values of ≤2 were more likely to be assigned to males than females. In contrast, RPS values of 3 or 4 were more likely to be assigned to females than males. This suggests that high level persistence of the epiphyseal scar is more likely to occur in females than males.

This, therefore, indicates that bone remodelling may occur to a greater extent in male individuals than females.

The statistical significance of the variation in the assignment of RPS values between the three regions of the proximal tibia in both sex cohorts was calculated through the application of a series of one-way ANOVA.

Table 7.14: Statistical significance of inter-region variation in Regional Persistence Scores in the proximal tibia in females and males

	Medial v Central	Central v Lateral	Medial v Lateral
Female	<0.001	<0.001	<0.001
Male	<0.001	0.018	<0.001

The results, presented in Table 7.14, show that with the exception of the variation between the central and lateral regions in male individuals, all interactions were highly statistically significant. Although the variation between central and lateral thirds of the bone in males was still statistically significant, the significance of this relationship was less than others in either sex. These results suggest that in females, bone remodelling and its influences are highly variable across the proximal tibia. Although this observation holds true in the male sample, the decrease in statistical significance of the variation observed between the central and lateral thirds of the bone compared with the medial and central regions suggests that bone remodelling within the central and lateral regions of the proximal tibia may occur at more similar rates than observed between other regions of the bone.

The results of these analyses show that variation in the persistence of the epiphyseal scar exists between regions of the bone both within and between sexes. To examine the relationship between region of the bone and the persistence of the epiphyseal scar further, a series of GLM analyses were undertaken, facilitating the analysis of region specific data in the context of chronological age, sex, side of the body and region of the bone. The results of these analyses are presented in Table 7.15.

Table 7.15: Results of the General Linear Model analyses for regional variation in the persistence of the epiphyseal scar in the proximal tibia

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	<0.001	0.044	0.029	2.9%
Sex	<0.001	0.022	0.022	2.2%
Side	<0.001	0.019	0.019	1.9%
Region	<0.001	0.062	0.061	6.1%
Age*sex	0.001	0.098	0.067	6.7%
Age*side	0.015	0.090	0.059	5.9%
Sex*side	0.528	0.042	0.040	4%
Region*side	0.312	0.083	0.080	8%
Region*sex	0.269	0.086	0.084	8.4%
Region*age	0.826	0.131	0.086	8.6%
Age*sex*side	0.527	0.155	0.096	9.6%
Region*side*sex	0.033	0.110	0.105	10.5%
Region*side*age	0.566	0.205	0.117	11.7%
Region*sex*age	0.756	0.211	0.123	12.3%
Region*sex*age*side	0.660	0.320	0.155	15.5%

This analysis suggests that when considered independently, all variables considered in this study exhibit statistically significant relationships with RPS ($P<0.001$) (Table 7.15). Within the independent factors, the strongest relationship was observed between region of the bone and RPS ($R^2=0.061$). Further analyses yielded only three additional statistically significant interactions between RPS value and the factors considered in this study. Although both age and sex; and age and side exhibited statistically significant relationships with RPS, the model of region, side and sex was found to explain the greatest percentage of variation in the assignment of RPS value ($P=0.033$; $R^2=0.105$). The relationship between region and RPS value was not found to be statistically significant when variation attributable to the other factors examined in this study was taken into account ($P=0.660$).

This study suggests that localised influence on remodelling occurs within the proximal tibia which results in variation in the persistence of the epiphyseal scar. The results obtained through these analyses show that although partially attributable to the factors included in this study, the majority of variation in the persistence of the epiphyseal scar in the proximal tibia cannot be explained by factors relating to the age, sex, side of the body or region of the bone under consideration.

7.3 Discussion of the persistence of the epiphyseal scar in the proximal tibia

7.3.1 Discussion of the intra-observer and inter-observer analysis in the proximal tibia

This study found that the variation between TPS values assigned by a single observer on two occasions was not statistically significant in either female or male individuals, however due to the statistical power of these analyses failing to reach the threshold value of 0.8, there is an increased risk of a Type II error (Cohen, 1992). Therefore, although these results suggest that the scoring system used to assess the persistence of epiphyseal scars in the proximal tibia may be consistent when applied on multiple occasions, they cannot be considered conclusive. In addition, no statistically significant difference was found between the TPS values assigned to females and males within the intra-observer sample.

Within the female sample, 80% of TPS values assigned at the second attempt were within 2 scores of those assigned at the first attempt. In male individuals, the percentage intra-observer agreement increased to 83.33%. As assessment of the female sample was completed prior to that of the male sample, these results suggest that experience in the application of the scoring system to a specific region may influence positively the level of intra-observer consistency. This finding is consistent with the beneficial effect of training on levels of intra-observer agreement observed in multiple age estimation studies (Rajan *et al.*, 2011).

To maintain the probative value of any resulting evidence, it is imperative that any method of anthropological assessment is applied consistently by multiple observers. This study found that the TPS values assigned by three observers did not differ significantly in either the female or male samples. Although this provided a statistical measure of the consistency between individuals, this analysis did not explain the variation between individual pairs of observers. It was therefore deemed appropriate to calculate the percentage agreement between observers. This also facilitated the quantification of variation between observers of different levels of experience in radiographic interpretation. These analyses found that the greatest percentage agreement in both sex samples was between the assessments of observers 2 and 3, where percentage agreement values of

100% and 83.33% were obtained for females and males respectively. As observers 2 and 3 were those with the highest levels of experience in radiographic interpretation, these results suggest that prior knowledge and experience in the analysis of x-ray images may be beneficial to the consistent assignment of TPS values. The lowest observed percentage agreement of 66.67% was found between the TPS values assigned to female individuals by observers 1 and 2, representing those individuals with the lowest and highest levels of experience in radiographic interpretation respectively. Within the male sample, an equal percentage agreement was observed between the assessments of observers 1 and 2 and observers 1 and 3. Further analysis showed that the variations between TPS values assigned by multiple observers were not statistically significant. This finding is consistent with the reported benefits of experience on the level of inter-observer agreement (Rajan *et al.*, 2011).

When the inter-observer percentage agreements obtained from the analysis of the female sample were compared with those derived from the male sample, it was observed that a higher percentage agreement was achieved in the female sample in all observer interactions. As all observers conducted their assessments of the female sample prior to those of the male sample, this cannot be attributed to inexperience in the application of the scoring system in this region as the expected result would be the reverse of that observed.

The results of the intra-observer and inter-observer analyses suggest that while the level of experience in radiographic interpretation may not influence positively the degree of consistency between observers to a significant degree, some knowledge and prior experience in general radiographic interpretation is important in the consistent application of the scoring system.

7.3.2 Discussion of the overall persistence of the epiphyseal scar in the proximal tibia

The proximal tibia, as a constituent part of the knee joint, has been included in several radiographic methods of age estimation (Pyle and Hoerr, 1969; O'Connor *et al.*, 2008; Cameriere *et al.*, 2012; O'Connor *et al.*, 2012). In their approach to age estimation from the knee, O'Connor *et al.* (2008) applied a scoring system to quantify the maturation of the proximal tibia and distal femur. Within the final

stage of maturation, it is noted that through osseous remodelling, trabecular continuity has been achieved across the former metaphyseal region, removing any trace of diaphyseal/epiphyseal demarcation; although with the inclusion of the caveat that a thin epiphyseal scar may remain in some individuals (O'Connor *et al.*, 2008). In contrast, the terminal stage of the scoring method applied by Cameriere *et al.* (2012) is only considered to have been achieved once all trace of the epiphyseal scar is obliterated. The divergent claims of these methods relating to the epiphyseal scar summarise the discourse which surrounds the importance of this feature and its place within the context of forensic age estimation.

This study found that a degree of persistence of the epiphyseal scar in the proximal tibia was observed in 98.05% of females and 97.74% of males. Although the total persistence rate observed in females and males differed by less than 1%, the inter-sex variation in the assignment of TPS was statistically significant. Neither female nor male data sets were normally distributed; however the distributions of these samples were statistically equivalent. This suggests that bone remodelling within the proximal tibia may be influenced by factors associated with the sex of the individual.

From the viewpoint of the forensic practitioner, perhaps the most crucial factor which may exert an effect on the long term behaviour of the epiphyseal scar is chronological age, as it is for the purpose of age estimation that this feature has previously been employed (Cameriere *et al.*, 2012). Initial analysis of the data in respect of the association between mean chronological age and persistence of the epiphyseal scar suggested that an inverse relationship may exist between chronological age and the persistence of the epiphyseal scar in the distal femur in both males and females.

As the characteristic of the epiphyseal scar that is related to chronological age, further investigations were undertaken to assess the relationship between maximum obliteration of the feature and chronological age. This was compared with the relationship between persistence of at least two thirds of the epiphyseal scar (TPS≥9) and chronological age. The results of these analyses showed that in both sex samples, the percentage of individuals to whom TPS values of ≥9 were

assigned displayed a moderate inverse relationship with increasing chronological age. In contrast, the relationship between maximum obliteration (TPS 0) and increasing chronological age showed a weak positive trend. In the cases of both the TPS 0 and TPS ≥9 cohorts, the coefficient of determination was higher in females than males, indicating that chronological age may exhibit a stronger influence on the remodelling of the epiphyseal scar in females than males. These analyses also show that complete obliteration of the epiphyseal scar is unlikely to be related to the chronological age of the individual in either sex.

Although initial analyses indicate that factors relating to chronological age and/or biological sex influence bone remodelling and therefore persistence of the epiphyseal scar in the proximal tibia, it was necessary to consider the potential variation attributable to these factors independently and as covariables. This study observed that when considered as an independent variable, chronological age displayed a statistically significant relationship with TPS; however, the variation in chronological age was found to explain only 2.7% of variation in TPS. Consequently, it is suggested that factors other than chronological age may exert an influence on the degree of retention of the epiphyseal scar. Subsequent analyses showed that although sex and side of the body both exhibited statistically significant relationships with TPS, the variation attributable to sex and side of the body explained only 4.5% and 3.6% of variation in TPS respectively.

These results indicate that when considered independently, biological sex, or factors with which it is associated, rather than chronological age exerts the strongest influence on remodelling of the epiphyseal scar in the proximal tibia. The variation in the persistence of the epiphyseal scar between females and males could be related to numerous factors which, through direct or indirect means, alter the rate of bone remodelling within the proximal tibia. These may include effects arising from variations in the levels of circulating oestrogens, body mass, physical activity, pregnancy and lactation in females, and nutrition (Mack and Vogt, 1971; Goldsmith, 1975; Hopkinson *et al.*, 2000; Compston, 2001; Karlsson *et al.*, 2001; Egan *et al.*, 2006). These analyses also showed that side of the body may influence the persistence of the epiphyseal scar in this anatomical region. This was supported by the results of subsequent analyses which showed statistically

significant degrees of intra-sex and inter-sex variation in the assignment of TPS values. The variation in the persistence of the feature between sides of the body may indicate that functional dominance of one side over the other may result in an alteration to the rate of bone remodelling within the favoured limb.

It is reported within the literature that approximately 90% of individuals are right-handed (Porac *et al.*, 1980; Cuk *et al.*, 2001; Blackburn, 2011). Unlike the upper limb however, there is a paucity of literature relating to bilateral asymmetry in the functional dominance of the lower limb; although a pattern of crossed-symmetry, whereby dominance of the lower limb occurs on the contralateral side to that of the upper limb, has been described (Auerbach and Ruff, 2006; Blackburn, 2011). Based on this premise, it would be expected that a high proportion of individuals would exhibit left-side functional dominance in the lower limb, from which greater mechanical loading would occur. The effect of mechanical loading on the stimulation of bone remodelling has been widely discussed, particularly in relation to the mechanostat principle, whereby the rate of bone remodelling increases until the structural competence of the skeleton matches the functional demands to which it is exposed (Frost, 1987; 1998b; Frost *et al.*, 1998; Frost, 2003; Hughes, 2010). According to the pattern of crossed-symmetry, this would lead to an increased level of obliteration of the epiphyseal scar in the left lower limb. This study however suggests that higher levels of bone remodelling in the region of the epiphyseal scar occur in the right limb, suggesting that this side of the body is under a higher degree of mechanical stimulus than the left side. This may be related to an increase in muscle mass or weight distribution as a result of preferential use of this limb.

When considered as independent variables, chronological age, sex and side of the body were all observed to exhibit statistically significant relationships with the degree of retention of the epiphyseal scar; however these factors may not be considered truly independent due to the complex interactions that exist between them. The analysis of these factors as covariables suggested that only the interaction between chronological age and sex exhibited a statistically significant relationship with TPS. The variation intrinsic to this interaction was found to account for 10.1% of variation within the assignment of TPS (Table 7.8). This

finding supports those of previous analyses which suggest that factors related to chronological age and biological sex influence the rate of bone remodelling in the proximal tibia, and consequently impact on the level of persistence of the epiphyseal scar within this region.

Although these analyses begin to illuminate the mechanism by which obliteration of the epiphyseal scar may occur, to further elucidate the processes contributing to obliteration of the feature, the persistence of the epiphyseal scar within the proximal tibia was examined in discrete regions. Through such analysis, patterns in the obliteration and persistence of the epiphyseal scar were examined and hypotheses regarding the obliteration were formulated.

7.3.3 Discussion of the regional variation in persistence of the epiphyseal scar within the proximal tibia

Due to the clinical origin of the radiographs included in this study, it was not possible to assess the influence of extrinsic variables on the persistence of the epiphyseal scar. As such, it was necessary to glean as much information as possible relating to the variation within the epiphyseal scar to facilitate the formulation of hypotheses relating to its aetiology and function in adult individuals.

Initial analysis showed that in both sex cohorts, the highest and lowest mean RPS values occurred in the medial and lateral thirds of the proximal tibia respectively. In addition, in all three regions of the bone, the mean RPS values calculated for female individuals were greater than those found in the male sample. Further analysis showed that in both sex cohorts, the lowest percentage of individuals in whom maximum obliteration of the epiphyseal scar occurred was observed in the medial third of the bone. Similarly, the lateral third exhibited the highest percentage of individuals where this was noted in both females and males. It was also found that in both sex cohorts, the medial and central thirds of the bone exhibited the highest percentage of individuals to whom RPS values of 3 and 4 were assigned respectively. For both these values, the lateral third exhibited the lowest percentage of individuals represented by the cohorts. These findings suggest that in the proximal tibia, remodelling occurs at the greatest rate within the lateral third of the bone; thus supporting the observation made by Pyle and

Hoerr (1969) who reported that the epiphyseal scar first disappeared in the area distal to the lateral tibial plateau.

This study also found that in all regions, a higher percentage of females than males were assigned RPS values of 3 or 4. Within the lateral third of the bone, this pattern was extended to include individuals to whom an RPS value of 2 was assigned. These results support the proposition that factors relating to the sex of the individual influence the rate of remodelling within the proximal tibia. The similarity in the pattern of persistence and obliteration observed between sex cohorts however suggests that this pattern is influenced by localised functional factors, for example the degree of mechanical loading to which the lateral tibial plateau is exposed or through forces related to the articulation with the proximal fibula.

It was apparent from these analyses that variation in the persistence of the epiphyseal scar was present across the proximal tibia. This study suggests that the rate of bone remodelling within this area increases in a medial to lateral direction resulting in statistically significant degrees of variation in the persistence of the epiphyseal scar between the medial, central and lateral thirds of the bone irrespective of sex. The least significant degree of variation between two regions was observed in the interaction of the central and lateral thirds in the male sample. This suggests that in males, the influences on bone remodelling within these regions are more similar than those found in the medial third of the bone.

Although the findings of this study are suggestive of localised influences on bone remodelling and the concomitant effects on the persistence of the epiphyseal scar, it was necessary to account for any variation attributable to the chronological age, sex, region and side of the body. Analysis of the relationships between these factors and RPS found that when considered independently, all four variables exhibited statistically significant relationships with RPS; however the strongest relationship was observed between region and RPS. Subsequent analyses yielded only three additional statistically significant relationships with RPS. The strongest of these models included region, sex and side of the body and explained 10.5% of variation in the assigned RPS value (Table 7.15). This suggests that within the

proximal tibia, the rate of bone remodelling and the subsequent effects on the persistence of the epiphyseal scar are influenced by locally acting factors which are associated with the sex of the individual and side of the body.

Based on the findings of this study, it is proposed that a mechanism related to the functional loading of the proximal tibia may result in an alteration to the rate of bone remodelling within specific areas of the bone and that this may exert a greater influence on the persistence of the obliteration of the epiphyseal scar in the lateral third of the bone than observed in the medial or central thirds of the proximal tibia. The magnitude of force to which the lateral third of the proximal tibia is exposed is reported to be less than that of the medial compartment (Johnson *et al.*, 1980a; 1980b; Hsu *et al.*, 1990; Hurwitz *et al.*, 1998; Tsuji *et al.*, 2001; Eckstein *et al.*, 2009); however due to the convex geometry of the lateral tibial plateau in the sagittal plane, the contact area of the lateral compartment of the knee is smaller than that of the medial side. Consequently, it is suggested that although the total load is less, the force per unit area may be greater in the lateral third of the proximal tibia. This hypothesis is supported by the findings of Koo and Andriacchi (2007) who, in their study of articular cartilage thickness, observed higher joint pressures in the lateral compartment of the knee than the medial compartment. This increase in pressure, in addition to stimulating growth of the articular cartilage, could induce an increase in bone remodelling and trabecular formation within the most proximal aspect of the lateral tibial plateau (Koo and Andriacchi, 2007). This may explain the findings of Khodadadyan-Klostermann *et al.* (2004) who observed that in successive 7mm slices of the proximal tibia, BMD progressively decreased in a diagonal pathway from the posterior-lateral to the anterior-medial regions of interest. The potential influence of soft tissue biomechanics on bone remodelling and BMD is also considered by these authors (Khodadadyan-Klostermann *et al.*, 2004). In consideration of the distribution of force, it is necessary to consider the valgus or varus angle of the femur as this may alter the trajectory of load transfer through the knee joint. The relationship between varus deformity at the knee and an increased rate of development of osteoarthritis has been reported (Brouwer *et al.*, 2007). This suggests that an increased varus angle alters the pattern of load distribution through the knee. As

this study was based on a clinical sample, the degree of femoral torsion or varus or valgus deformation was not known, however the potential influence should be considered.

It has also been reported that during the normal gait cycle, a lesser degree of anterior-posterior translation in the tibio-femoral contact point occurs in the lateral compartment than the medial compartment (Koo *et al.*, 2011). This suggests that a narrower distribution of force in the lateral tibial plateau will occur during normal walking; and as a result, an increase in the localised application of mechanical load may occur within the lateral compartment relative to that observed in the medial tibial plateau.

Under the mechanical loading paradigm, the variation in the assignment of RPS values between females and males could be attributable to differences in the anthropometric characteristics between the sexes which could potentially include total body mass and lean muscle mass (Janssen *et al.*, 2000; Lee *et al.*, 2000). Male individuals, as a function of higher levels of testosterone, possess a larger total body mass and lean muscle mass than females (Janssen *et al.*, 2000; Abe *et al.*, 2003). During this study, it was observed that male individuals exhibited higher levels of obliteration of the epiphyseal scar in all three regions when compared to females. This is indicative of an increased degree of bone remodelling within this sex cohort. This finding is supported by the literature in which it has been shown through the analysis of markers of bone turnover that males exhibit a higher rate of bone remodelling than females (Henry and Eastell, 2000); however this may be counteracted by the increase in relative bone size and body mass which confer protective effects on BMD. As the function of the lower limb is not contingent upon the sex of the individual, this study suggests that the variation in the persistence of the epiphyseal scar observed between females and males may be related to variations in the magnitude of mechanical loading to which the limb is exposed.

Based on the statistically significant model with the greatest explanatory power, the results of the GLM analyses suggest that in addition to biological sex, side of the body may be a factor in the level of persistence of the epiphyseal scar. Although initially suggestive of a potential role of functional limb dominance, this result may

have occurred as a consequence of the strength of the relationship between biological sex and RPS values. When previous analyses are considered, the relationship of region and side with RPS was not statistically significant, neither was that of side and RPS when assessed as an independent variable. It is therefore concluded that although the strongest statistically significant output of the GLM analysis includes side of the body, the variation in the persistence score attributable to this factor is small.

8 Persistence of the Epiphyseal Scar in the Distal Tibia

8.1 Sample distribution

The sample distribution by age, sex and side of the body is presented in Table 8.1.

Table 8.1: Distribution of the sample used in the analysis of the distal tibia according to chronological age, biological sex and side of the body

Age (Years)	Female Left	Female Right	Male Left	Male Right
20	5	5	5	5
21	5	5	5	5
22	5	5	4	5
23	5	5	5	4
24	5	5	5	5
25	5	5	5	5
26	5	5	5	5
27	5	5	5	5
28	5	4	5	5
29	5	5	5	5
30	5	5	5	5
31	5	5	4	5
32	4	4	5	5
33	5	5	3	5
34	4	5	5	2
35	5	5	4	5
36	5	5	5	5
37	5	5	5	3
38	5	5	5	5
39	5	5	5	5
40	5	5	5	5
41	4	5	5	5
42	5	5	5	5
43	5	5	5	5
44	5	5	5	5
45	5	5	5	5
46	5	4	4	5
47	5	4	5	5
48	5	5	5	5
49	5	5	5	5
50	5	4	5	5
Total	**152**	**150**	**149**	**149**

8.2 Results

8.2.1 Intra-observer analysis

Initially, a series of one-way ANOVA were carried out to determine the consistency between assignments of TPS made by a single individual on two occasions. These

analyses suggested that there was no significant difference between the scores assigned on the first and second attempts in either the females (P=0.428) or males (P=0.199). The variation between the TPS assigned to females and males was determined through the application of ANOVA. This analysis suggested that there was no statistically significant difference in the TPS assigned (P=0.124). Analysis of the data obtained from the intra-observer assessments suggested that 66.67% of scores assigned to females and 80% of scores assigned to males were within two scores of those assigned at the first attempt. Figure 8.1 presents the variation between first and second observations as a percentage of the intra-observer sample.

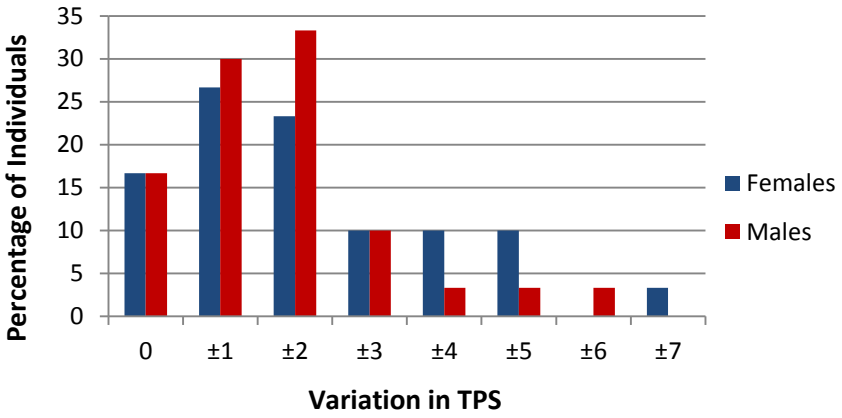

Figure 8.1: Intra-observer variation in Total Persistence Score assigned to the distal tibia according to biological sex

The statistical significance of the interactions between biological sex; round of assessment and TPS were analysed using a univariate GLM analysis, the results of which are presented in Table 8.2.

Table 8.2: Results of the General Linear Model analysis of intra-observer variation in the distal tibia

Factor (s)	P-Value	R	R^2	Adjusted R^2	% Variation Explained
Sex	0.071	0.21	0.044	0.028	2.8
Round	0.847	0.00	0.000	0.008	0.8
Sex*Round	0.192	0.24	0.059	0.026	2.6

These data suggest that the relationship between the TPS and round of assessment is not statistically significant (P=0.847). As analyses were conducted on images from both sex cohorts, it was necessary to consider the influence of the combined

effects of biological sex and round of assessment on the assignment of TPS. The effect of variation between repeated assessments, when the variation attributable to biological sex was taken into consideration, was found to explain only 2.6% of variation in the TPS assigned during the intra-observer analysis. The results of the GLM suggest that any variation which exists between the TPS assigned to individuals in successive rounds of assessment is not statistically significant.

8.2.2 Inter-observer analysis

Initially, a one-way ANOVA was carried out to determine whether statistically significant variation existed between the TPS assigned to the female and male sample. The results of this analysis suggested that any variation between the TPS assigned to females and males was not statistically significant (P=0.597).

The variation in TPS assigned by multiple observers was calculated using a one-way ANOVA. The results of these analyses suggested that the variation in TPS assigned by multiple observers was not statistically significant in either the female (P=0.384) or male (P=0.696) samples. To examine the degree of inter-observer consistency further, the percentage agreement between observers was calculated and is presented in Table 8.3.

Table 8.3: Inter-observer percentage agreement in Total Persistence Score in the distal tibia

Sex	Obs 1 v Obs 2	Obs 1 v Obs 3	Obs 2 v Obs 3
Female	90.00	86.67	83.33
Male	73.33	83.33	83.33

The statistical significance of the variation in the TPS values assigned by the three observers was calculated through the application of a series of one-way ANOVA. The results of these analyses, presented in Table 8.4, show that no interactions were statistically significant. Although not statistically significant, the interaction between observers 1 and 3 included the highest degree of variation in the male sample. Within the female sample, the most variable relationships were found to be those between observers 1 and 3 and 2 and 3.

Table 8.4: Statistical significance of inter-observer variation in the assignment of Total Persistence Scores in the distal tibia according to biological sex

Sex	Obs 1 v Obs 2	Obs 1 v Obs 3	Obs 2 v Obs 3
Female	0.958	0.232	0.232
Male	0.218	0.071	0.612

The maximum variation between assigned TPS values within the female samples was ±4 scores. Although this degree of discrepancy was observed in all paired comparisons, the highest frequency of this level of variation occurred in the comparison of the responses of observers 2 and 3. Within the male sample, the greatest variation between TPS scores was ±5 and was found in the interaction between observers 1 and 2. In contrast to the results derived from the analysis of the female sample, the interaction between observers 2 and 3 provided the narrowest range of variation, with the greatest discrepancy being ±3 scores.

The statistical relationships between observer, biological sex and TPS were examined through the application of GLM analyses, the results of which are presented in Table 8.5.

Table 8.5: Results of the General Linear Model analysis of inter-observer variation in the distal tibia

Factor(s)	P-Value	R	R^2	Adjusted R^2	% Variation Explained
Sex	0.635	0.031	0.001	-0.004	0
Observer	0.105	0.158	0.025	0.014	1.4
Observer*Sex	0.616	0.179	0.032	0.004	0.4

These results suggest that neither the independent or combined effects of variation attributable to observer or biological sex exhibit a statistically significant relationship with TPS value.

8.2.3 Main data analysis

Initial analysis of the data was undertaken to determine the distribution of the sample according to TPS in sex specific groups (Figure 8.2). This study found that 92.72% of females and 92.95% of males were observed to exhibit a degree of preservation of the epiphyseal scar at the distal tibia.

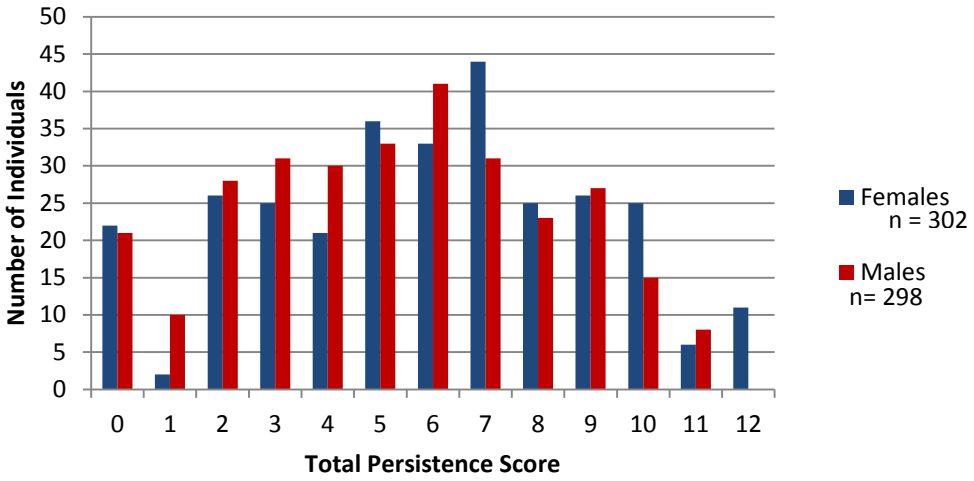

Figure 8.2: Distribution of the distal tibia study sample according to biological sex and Total Persistence Score

The results of a Shapiro-Wilk normality test showed that neither the female (W-statistic=0.971) nor male (W-statistic=0.968) samples were distributed normally.

Prior to determining the significance of the influence of the age, sex and side of the body on the TPS assigned to an individual, the mean age of the individuals to whom each persistence score was attributed was calculated. These data are presented in Table 8.6 and Table 8.7 in females and males respectively.

Table 8.6: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal tibia in female individuals

Total Persistence Score	Mean (years)	Maximum (years)	Minimum (years)
0	40.37 (n=27)	50	21
1	42.00 (n=2)	50	34
2	38.04 (n=26)	50	23
3	37.08 (n=24)	48	26
4	36.57 (n=21)	50	20
5	36.28 (n=36)	50	23
6	33.21 (n=33)	49	22
7	33.95 (n=44)	49	20
8	34.44 (n=25)	49	20
9	31.12 (n=26)	48	20
10	31.08 (n=25)	49	20
11	28.83 (n=6)	35	21
12	29.73 (n=11)	50	20

The relationship between mean chronological age and TPS value was assessed through the calculation of the net difference between the mean chronological age in cohorts 2 and 10. As these TPS values represent the maximum and minimum

level TPS values that were common to both sexes where n>10, they provide a scale against which the trend in chronological age may be assessed. In the female data set, a net difference of 6.96 years was observed between the mean chronological ages assigned to TPS values 1 and 10. This is contrasted with a difference of 6.36 years in the male sample. These data suggest that there may be an inverse relationship between chronological age and ascending TPS values in the distal tibia, as would be expected if the level of persistence or obliteration of the epiphyseal scar is related to chronological age.

Table 8.7: Mean, maximum and minimum chronological ages for individuals represented by each Total Persistence Score in the distal tibia in male individuals

Total Persistence Score	Mean (year)	Maximum (years)	Minimum (years)
0	33.19 (n=21)	49	23
1	35.50 (n=10)	47	20
2	37.36 (n=28)	49	23
3	35.10 (n=31)	50	20
4	38.23 (n=30)	50	20
5	35.39 (n=33)	49	20
6	37.20 (n=41)	50	20
7	35.39 (n=31)	50	20
8	33.30 (n=23)	48	20
9	31.74 (n=27)	50	20
10	31.00 (n=15)	50	20
11	29.00 (n=8)	48	21
12	--	--	--

To examine the distribution of TPS values among the sample populations further, the percentage of individuals within each age group for whom TPS values ≥9 or 0 were assigned were calculated. These TPS values correspond to persistence of at least two-thirds of the epiphyseal scar and complete obliteration of the feature respectively. The results of these analyses are presented in Figure 8.3 and Figure 8.4 for females and males respectively.

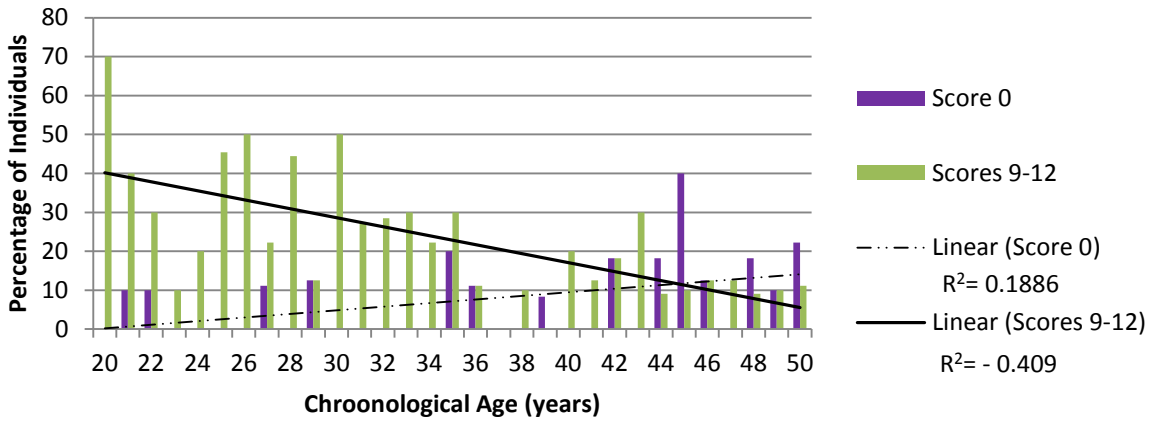

Figure 8.3: Percentage of female individuals exhibiting complete obliteration or maximum persistence of the epiphyseal scar in the distal tibia according to chronological age

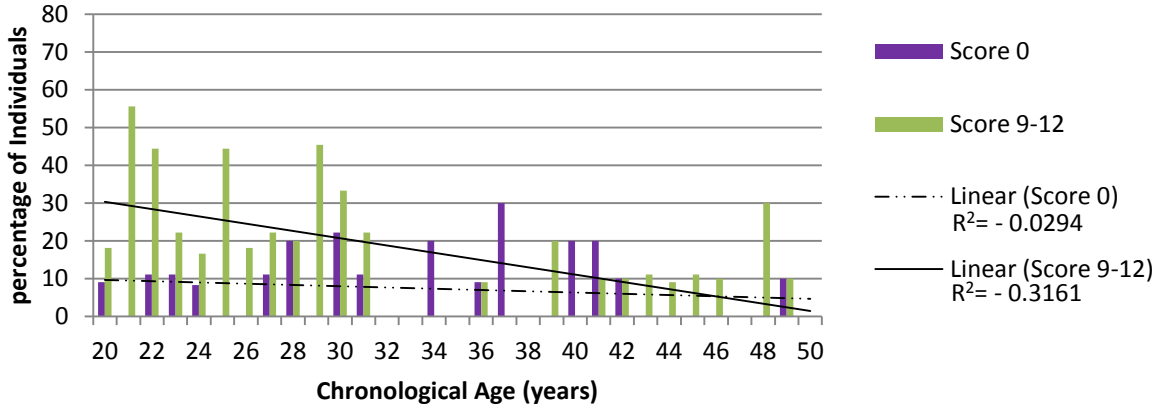

Figure 8.4: Percentage of male individuals exhibiting complete obliteration or maximum persistence of the epiphyseal scar in the distal tibia according to chronological age

From the analyses presented in Figure 8.3 and Figure 8.4, several trends were observed in the distribution of the data. Overall, a moderate negative trend (R^2=-0.409) was noted in the percentage of female individuals assigned a TPS value ≥9 according to chronological age. A similar, though weaker, trend was observed in the male sample where a linear regression of TPS against chronological age showed there to be an inverse relationship between the factors (R^2=-0.3161). Within the female sample, a positive trend in the percentage of individuals in whom maximum obliteration of the epiphyseal scar was observed (R^2=0.1886). In contrast, within the male sample, a weak negative trend in the percentage of individuals in whom maximum obliteration of the epiphyseal scar was recorded (R^2=-0.0294).

To determine the influence of intrinsic factors on the persistence of the epiphyseal scar, the raw data obtained from the analysis of radiographs was analysed using a GLM analysis. This facilitated the examination of the relationships between factors including chronological age, biological sex and side of the body, and TPS.

Table 8.8: Results of the General Linear Model analyses in the distal tibia

Factor(s)	Significance	R	R^2	R^2 Adjusted	% Variation Explained
Age	<0.001	0.35	0.123	0.076	7.60%
Sex	0.012	0.10	0.011	0.009	0.90%
Side	0.146	0.06	0.004	0.002	0.20%
Age*sex	0.01	0.46	0.21	0.12	12.00%
Age*side	0.137	0.43	0.184	0.092	9.20%
Sex*side	0.001	0.57	0.32	0.27	27%
Age*sex*side	0.204	0.58	0.337	0.166	16.60%

The results of the GLM analysis, presented in Table 8.8, suggest that there is a significant relationship between chronological age and TPS; however, chronological age was found to account for only 7.6% of variation within the TPS when considered as an independent variable. The only other factor to display a significant relationship with TPS when considered independently was biological sex (P=0.012). Despite the high degree of statistical significance attributed to the influence of biological sex on TPS, it was found to account for only 0.9% of variation within the TPS. When assessed as an individual variable, side of the body was not found to have a significant relationship with TPS (P=0.146).

Due to the interactions between sex and chronological age in terms of skeletal maturation and maintenance, it was necessary to examine the relationships between TPS and its covariant factors. These interactions showed that when age and sex were considered as covariables, variation within this interaction accounted for 12% of variation within the sample, thereby accounting for a greater proportion of the variation than when considered as independent variables.

Although side of the body from which the radiograph was taken was not found to exert a significant influence on TPS when analysed as an independent variable, the interaction between sex and side of the body was found to exhibit a significant relationship with TPS (P=0.001). This interaction accounted for 27% of variation within the sample data. Despite the relationship between chronological age and

biological sex, and side of the body and biological sex being significant, the interaction between chronological age and side of the body was not significant (P=0.137).

Further analysis of the interaction between sex and side of the body was undertaken through the application of a series of ANOVA. The results of these analyses are presented in Table 8.9.

Table 8.9: Reciprocal table of the statistical significance of variation in the assignment of Total Persistence Score in the distal tibia according to biological sex and side of the body

	Female Left	Male Right
Female Right	0.144	0.433
Male Left	<0.001	<0.001

These results suggest that there was no statistically significant difference between the TPS assigned to female left and right distal tibiae (P=0.144). Within the male sample however, there was a statistically significant degree of variation between the TPS values assigned to the left and right sides of the body (P<0.001). Within the inter-sex comparisons, there was no statistically significant variation between the TPS values assigned to right distal tibiae in females and males (P=0.433). A statistically significant degree of variation was observed between the TPS values assigned to the left distal tibiae in females and males (P<0.001).

These data were further analysed and the mean TPS value assigned to each sex and side specific cohort was calculated. These results, presented in Table 8.10, show that the highest mean TPS value was observed in the female left distal tibial cohort while the lowest mean TPS value occurred in the male left distal tibial cohort.

Table 8.10: Mean total persistence score in the distal tibia according to biological sex and side of the body

	Left	Right
Female	6.12	5.67
Male	4.69	5.86

To assess the variation in persistence of the epiphyseal scar across the expanse of the bone, RPS values were calculated for the medial, central and lateral thirds of the bone. Initially, the mean RPS assigned to each region of the bone was

calculated for females and males. These data, presented in Table 8.11, suggest that in females, the greatest mean persistence of the epiphyseal scar was observed in the lateral region of the bone. In males however, the highest mean RPS was found in the central third of the bone. This analysis also showed that the medial third of the distal tibia exhibited the lowest mean RPS values in both sex cohorts.

Table 8.11: Mean Regional Persistence Scores for females and males in the distal tibia

	Medial Region	Central Region	Lateral Region
Female	1.20	1.97	2.73
Male	1.37	2.04	1.86

Further analysis of the RPS data was conducted through the calculation of the distribution of RPS values according to region of the distal tibia to which they were assigned. The results of this analysis are presented in Table 8.12 and Table 8.13 for females and males respectively.

Table 8.12: Percentage distribution of Regional Persistence Scores in the distal tibia in female individuals

n=302	Medial	Central	Lateral
0	30.46	14.57	11.59
1	36.75	21.52	6.95
2	21.85	30.46	19.54
3	4.30	19.21	20.53
4	6.62	14.24	41.39
Total	**100%**	**100%**	**100%**

Table 8.13: Percentage distribution of Regional Persistence Scores in the distal tibia in male individuals

n=298	Medial	Central	Lateral
0	17.45	13.09	28.86
1	37.92	18.12	14.77
2	34.56	34.90	20.13
3	10.07	19.46	13.76
4	0.00	14.43	22.48
Total	**100%**	**100%**	**100%**

These data show that in females, the highest incidence of assignment of RPS 0 occurred in the medial third of the distal tibia. In males, this was found in the lateral third of the bone. In addition, the medial third of the bone was observed to

exhibit the lowest percentage of individuals in whom maximum persistence of the epiphyseal scar was recorded in both sex cohorts. Although the percentage distribution of RPS 4 differed between sexes, the regional pattern in the assignment of this score was found to be the same. This showed that in both sex cohorts, the lateral third of the bone was most likely to be assigned a score of 4.

The statistical significance of the variation in the assignment of RPS scores between regions was calculated through the application of a series of ANOVA. The results of these analyses, presented in Table 8.14, show that within the female sample, statistically significant degrees of variations in the persistence of the epiphyseal scar were observed between all three regions of the bone. In males, no statistically significant variation was found between the central and lateral regions of the bone, however statistically significant variation was found between the medial and central; and medial and lateral regions.

Table 8.14: Statistical significance of inter-region variation in Regional Persistence Scores in the distal tibia in females and males

	Medial v Central	Central v Lateral	Medial v Lateral
Female	<0.001	<0.001	<0.001
Male	<0.001	0.105	<0.001

Further analyses of variance found that there was no statistically significant variation in the assignment of RPS values to the central region between sex cohorts (P=0.464), however statistically significant variation was observed in both the medial and lateral regions of the bone to a levels of 0.001 and ≤0.001 respectively.

To take into consideration the effect of biological sex, side of the body and chronological age on the variance between persistence scores of the three regions, a GLM analysis was undertaken. The results of this analysis are presented in Table 8.15.

These data show that while region of the growth plate appears to have a significant influence on the persistence score assigned (P<0.001), this relationship becomes insignificant when other factors affecting the data are included in the analysis. Region is maintained as a significant influence on persistence score when biological sex is considered as a covarying factor (P<0.001) and explains 13.6% of

variation within the sample. When combined with all variant factors within the analysis, the influence of region on persistence score becomes statistically not significant (P=0.933).

Table 8.15: Results of the General Linear Model analyses for regional variation in persistence of the epiphyseal scar in the distal tibia

Factor(s)	Significance	R^2	R^2 Adjusted	% Variation Explained
Age	<0.001	0.072	0.056	5.6%
Sex	0.001	0.006	0.006	0.6%
Side	0.059	0.002	0.001	0.1%
Region	<0.001	0.102	0.101	10.1%
Age*sex	<0.001	0.119	0.088	8.8%
Age*side	<0.001	0.113	0.081	8.1%
Sex*side	<0.001	0.018	0.016	1.6%
Region*side	0.726	0.104	0.101	10.1%
Region*sex	<0.001	0.138	0.136	13.6%
Region*age	0.307	0.204	0.161	16.1%
Age*sex*side	0.003	0.195	0.136	13.6%
Region*side*sex	0.183	0.152	0.147	14.7%
Region*side*age	0.975	0.263	0.179	17.9%
Region*sex*age	0.951	0.300	0.220	22%
Region*sex*age*side	0.933	0.414	0.262	26.2%

8.3 Discussion of the persistence of the epiphyseal scar in the distal tibia

8.3.1 Discussion of intra-observer and inter-observer analysis in the distal tibia

As this study represents the first specific examination of the persistence or obliteration of the epiphyseal scar in the distal tibia within a selected population, it is imperative that the scoring system itself is tested to determine its consistency and reliability when applied on multiple occasions or by multiple individuals. The intra-observer study, conducted as part of the wider investigation, determined that there was no significant difference between the TPS assigned on multiple occasions by the same individual in either sex cohort. It must be acknowledged however that due to the statistical power of the ANOVA analysis failing to reach the threshold value of 0.8, there is an increased risk that a Type II error occurred, resulting in the false acceptance of the null hypothesis. Although not conclusive, these results suggest that the method is statistically repeatable when applied by a single observer.

This study showed that 66.67% and 80% of TPS assigned to females and males respectively at the second attempt were within 2 scores of those assigned at the first round of assessment. The relatively low percentage agreement observed in the female sample is potentially attributable to the order in which the assessments were carried out. The female distal tibia was the first region in which the epiphyseal scar was examined. As the second round of assessments were carried out following the completion of all first round assessments, this could suggest that experience in the assessment of epiphyseal scars may exert an influence on the consistency of TPS values assigned by a single examiner on two occasions.

In addition to intra-observer variation, it was necessary to assess the consistency of the scoring system when applied by multiple observers. This study found there to be no statistically significant variation between observers in either sex cohort. As was observed in the intra-observer analysis, the statistical power of the ANOVA test did not reach the threshold of 0.8. Consequently, the likelihood of the occurrence of a Type II error increases. Therefore, although the results of this inter-observer study are indicative of consistency, they cannot be considered conclusive.

As this study utilised three observers, each with different levels of experience in radiographic interpretation, it was necessary to calculate the agreement between the various observers and the statistical significance of the variation in their assignments. This analysis found that the greatest percentage agreement in the female sample of 90% was observed between observers 1 and 2. This interaction was also found to exhibit the lowest level of inter-observer variation in assigned TPS values. The lowest percentage agreement in the female sample occurred between observers 2 and 3. Although not statistically significant, this interaction was found to exhibit a p-value equivalent to that observed in the interaction between observers 1 and 3.

Within the male sample, the percentage agreements between observers 1 and 3 and 2 and 3 were found to be equal. Further analysis however showed that the TPS values assigned by observers 1 and 3 in the male sample exhibited a higher degree of statistical significance. This indicates that although the percentage of

individuals for whom the assigned TPS values were within 2 scores of each other was found to be equivalent, the inter-observer consistency in the remaining assessments was greater between observers 2 and 3 than between observers 1 and 3. The lowest percentage agreement of 73.33% was found between observers 1 and 2.

The results obtained from this test of inter-observer reliability showed that the percentage agreement obtained from the analysis of female sample was consistently equal to or higher than that observed in the male sample. As the assessments on the female sample were conducted prior to those on the male sample, these results suggest that limited experience in the application of the method may not necessarily confer a greater level of inter-observer consistency. These results suggest that while the level of experience in radiographic interpretation or age estimation does not appear to exert a strong influence on the ability of the observer to employ the scoring system presented in this study to the distal tibia, some experience in these fields may lead to a greater level of inter-observer concordance.

8.3.2 Discussion of the overall persistence of the epiphyseal scar in the distal tibia

Unlike other regions of the body, the distal tibia, as a component of the foot and ankle has received little attention from research into skeletal age estimation. Prior to the results presented in Chapter 3 of this thesis, only one method of radiographic age estimation (Hoerr *et al.*, 1962) has been validated and found suitable for application in forensic practice (Hackman *et al.*, 2013). Although not specifically used as a maturity criterion within the radiographic atlas by Hoerr *et al.* (1962), the potential for the epiphyseal scar, in this text referred to as the "terminal line", to remain visible throughout life in both the tibia and fibula was noted. Despite the acknowledgement that this feature may remain in skeletally mature individuals, the temporal stability of the epiphyseal scar has not been a matter of specific examination (Hoerr *et al.*, 1962).

The results obtained by this study suggest that the epiphyseal scar is likely to persist to some degree in the region of the distal tibia in over 90% of individuals, irrespective of sex. Several trends were noted in the distribution of the data

among both the female and male samples, particularly in those individuals for whom no epiphyseal scar was observed (TPS 0). A moderate positive relationship between the percentage of female individuals represented by this cohort and chronological age was observed. This suggests that disappearance of the epiphyseal scar may be more likely in individuals of more advanced age. This finding was not repeated in the analysis of the data derived from male individuals where a slight inverse trend was identified in the relationship between chronological age and the maximum obliteration of the epiphyseal scar. Although this pattern does not follow the expected trend, the R^2 value obtained for the relationship between TPS 0 and increasing chronological age in male individuals was extremely weak. As only a small number of individuals were represented by TPS 0, the presence of an inverse relationship between complete obliteration of the epiphyseal scar and increasing chronological age observed in this study may not be representative of a larger sample size.

Further analysis showed that a strong negative trend existed between percentage of individuals observed to retain at least two thirds (TPS≥9) of the distal tibial epiphyseal scar and chronological age in both the female and male samples. Within the female sample, a reduction in percentage of individuals included within this TPS cohort of approximately 60% was observed compared with a decrease of approximately 20% in males as chronological age increased. Although this overall trend was observed in both sex samples, it was noted that a greater percentage of young females were represented by this TPS group compared with males of the same chronological age. This suggests that a greater proportion of the epiphyseal scar may be more likely to persist in female individuals.

The primary characteristic traditionally associated with the obliteration of the epiphyseal scar is chronological age, however due to the complexity of the interactions between factors which may act on bone remodelling and therefore the persistence of the epiphyseal scar, it was necessary to consider all factors both individually and as co-varying influences. This study suggests that a statistically significant relationship does exist between chronological age and TPS and therefore the obliteration of the epiphyseal scar, however this interaction was found to explain only 7.6% of variation in TPS (Table 8.8). Therefore while age

may influence the epiphyseal scar, the relationship is not of sufficient strength to warrant the application of the disappearance this feature as a criterion of age estimation from the distal tibia. In addition to chronological age, this study found a statistically significant relationship between biological sex and TPS and therefore the obliteration of the epiphyseal scar. Although the relationship between these factors was statistically significant, subsequent analyses showed that less than 1% of variation in obliteration of the epiphyseal scar is attributable to variation in biological sex (Table 8.8). The low degree of variation in TPS explained by this interaction suggests that although factors related to the biological sex of the individual may exert an influence on the degree of obliteration of the epiphyseal scar in the distal tibia, the pattern of persistence or obliteration of the feature is likely to be influenced by additional factors.

Several sex related factors, including levels of circulating systemic hormones and body mass, have been reported to influence the rate of bone formation and resorption, which as coupled actions, results in an alteration to the tempo of bone remodelling and turnover (Compston, 1992; Compston, 2001). The extent to which these factors influence the rate of bone remodelling often varies depending on the life stage of the individual. Consequently, it was necessary to consider the relationship between TPS and the combined influence of chronological age and sex. The results of this study suggest that a statistically significant relationship exists between chronological age and sex, and TPS. In addition to this, the variation inherent to this interaction was found to explain 12% of the variation in TPS and therefore the obliteration or persistence of the epiphyseal scar, representing a substantial increase in explanatory power compared with either factor when considered independently (Table 8.8).

Although statistically significant relationships were observed between TPS and age and sex as both independent factors and covariables, the low percentage of the variation in TPS explained by these variables suggests that other factors contribute to the degree of preservation of the feature. The results of this study suggested that there was no significant relationship between side of the body and TPS in this anatomical region. In addition, side of the body was found to explain only 0.2% of the variation observed in TPS (Table 8.8). As a result, it may be concluded that the

results of this study suggest that when considered as an independent variable, side of the body does not significantly influence the obliteration or persistence of the epiphyseal scar at the distal tibia.

Due to the difference in musculature between males and females and the increased likelihood of males to participate in strenuous activity, there may be an interaction between sex and side of the body which when combined, explains a greater percentage of variation in the degree of retention of the epiphyseal scar than either factor when considered independently. This study suggests that there is a statistically significant relationship between sex and side of the body. In addition to being highly significant, this relationship explained 27% of the variation in TPS (Table 8.8). Further analysis of the relationship between sex and side of the body showed that the data relating to the male left distal tibiae were statistically different from those derived from the analysis of the male right and female left distal tibiae. This indicates that variation attributable to side of the body observed in the overall analysis may be due to that found in the male left cohort. As a result of these analyses, it may be concluded that sex and side of the body represent the greatest influencing factors on the epiphyseal scar at the distal tibia. An individual may exhibit limb dominance, the discrepancy in weight distribution or muscle accretion created by this functional preference, may influence the degree of retention of the epiphyseal scar. It is suggested in the literature that the skeletal manifestations of functional dominance may be due to an increased degree of musculature in the dominant limb (Auerbach and Ruff, 2006). As a result of the increased muscle mass and strength observed in male individuals compared with females, the findings of this study could be attributable to a greater influence of functional dominance on the musculature of males relative to females and the subsequent effects of this on osseous remodelling and therefore the behaviour of the epiphyseal scar.

The muscularity of an individual may change through their life time as a result of the life-style changes associated with increasing age. Consequently, the influence of limb dominance on the epiphyseal scar could be altered by increasing chronological age. No statistically significant relationship between age and side of the body was found by this study.

8.3.3 Discussion of the regional variation in persistence of the epiphyseal scar within the distal tibia

It is apparent from the results obtained during this study that the degree of persistence of the epiphyseal scar in the distal tibia is influenced by factors other than those considered in this research. It is therefore necessary to consider other influences to which this region of the skeleton is exposed that may cause sufficient alteration to bone remodelling to elicit changes in the cancellous structure and subsequent obliteration of the epiphyseal scar.

Analysis of the epiphyseal scar in the medial, central and lateral regions of the distal tibia showed that in female individuals, the highest mean persistence of the epiphyseal scar occurred in the lateral third of the bone. In addition, this region was found to exhibit the highest percentage of individuals to whom RPS values of 3 or 4 were assigned and the lowest percentage of individuals in whom maximum obliteration of the epiphyseal scar had occurred. In male individuals, the highest mean persistence score was found in the central region of the distal tibia; however as observed in females, the highest percentage of individuals to whom an RPS value of 4 was assigned occurred in the lateral third of the bone; however the highest percentage of individuals in whom maximum obliteration of the epiphyseal scar was observed also occurred in this area of the bone. These results indicate that a greater level of persistence of the epiphyseal scar may occur in the lateral and central thirds of the distal tibia in females and males respectively.

Analysis of the regional persistence of the epiphyseal scar also showed that the lowest mean persistence score was attributed to the medial third of the distal tibia in both sex cohorts. Within this region, it was observed that a higher percentage of females than males exhibited maximum obliteration of the epiphyseal scar. In male individuals however, the percentage of subjects in whom no epiphyseal scar was found was lower than that observed in the lateral third of the bone. Within the medial region, it was also observed that complete persistence of the feature only occurred in female individuals; however a higher percentage of females than males were found to exhibit a maximal degree of obliteration of the epiphyseal scar. This study also found that this region of the bone also exhibited the highest percentage of individuals to whom an RPS value of 1 was assigned in both sex

cohorts. This suggests that in both sexes, a greater degree of bone remodelling occurs in the medial region than in either the central or lateral thirds of the bone, suggesting that complete persistence of the epiphyseal scar is unlikely to occur in this region of the distal tibia.

Further analysis showed that within the female sample, a statistically significant degree of variation existed between each of the three regions of the distal tibia. Within males however, no statistically significant variation was found between the RPS values assigned to the central and lateral thirds of the bone; however statistically significant differences were found between the medial and central; and medial and lateral thirds of the bone. In addition, it was found that no statistically significant variation existed between the RPS values assigned to females and males in the central third of the distal tibia. This finding was not repeated in the lateral and medial regions of the bone where statistically significant degrees of inter-sex variation were observed. These findings suggest that bone remodelling within the central third of the distal tibia is subjected to similar degrees of influence irrespective of sex. The persistence of the epiphyseal scar in this region may therefore be similar in both sex cohorts.

It is apparent from these findings that significant variation in the rate of bone remodelling occurs within the distal tibia in both females and males and that the obliteration of the epiphyseal scar is linked to factors other than chronological age or side of the body. The results of this study are suggestive of localised effects which are linked to the position within the epiphyseal scar and the sex of the individual. This proposition is supported by the results of GLM analyses which found that 13.6% of the variation in regional persistence of the epiphyseal scar was attributable to the combined variation in region of the bone and sex of the individual. It is suggested that the variability in the persistence of the epiphyseal scar in the distal tibia occurs as a result of the combined influence of multiple factors related to the distribution of mechanical loading through the ankle joint and as a result, the distal tibia.

The forces to which the ankle and therefore the distal tibia, is subjected may be generated through static or dynamic loading and may result in the transference of

a load equivalent to between two and four times the body weight of the individual (Kleipool and Blankevoort, 2010; Suckel *et al.*, 2010). As mechanical loading affects the rate of bone remodelling, the trajectory along which the load in the distal tibia is transmitted may be of importance in explaining the variation observed in the persistence of the epiphyseal scar across the expanse of the bone (Frost, 1996).

It is reported within the literature that force transmission through the tibiotalar joint primarily occurs through the antero-lateral aspect of the articular contact area (Suckel *et al.*, 2010); however parity in the distribution of force between the medial and lateral aspects of the bone has also been discussed (Bruns and Rosenbach, 1990). As mechanical loading is known to influence the rate of bone remodelling, it would be reasonable to suggest that the pattern of distribution of mechanical loading stated in the literature would be reflected in the observed persistence of the epiphyseal scar. The results of this study however suggest that the medial third of the distal tibia is exposed to the highest degree of mechanical loading in both sexes. It appears, however, that in this region, bone remodelling in female individuals may be exposed to greater levels of influence than males. In addition, this study suggests that in female individuals, bone remodelling within the lateral third of the distal tibia occurs as a slower rate than in other regions of the bone; and in male individuals, remodelling occurs at a similar rate to that within the central portion of the bone.

In addition to the role of weight bearing on the mechanical loading of the distal tibia, it is necessary to consider additional intracorporeal or extracorporeal factors which could impart a degree of mechanical loading on the bone or alter the trajectory of applied load transmission through the region.

The interosseous membrane of the leg is reported to play a role in the distribution of load between the tibia and fibula (Minns and Hunter, 1976). Situated on the lateral side of the tibia, the majority of fibres within the interosseous membrane of the leg pass from the lateral aspect of the tibia to the fibula in a proximal-distal direction, however some fibres do pass in the opposite direction (Vukičević *et al.*, 1980). This may suggest that the force applied to the lateral aspect of the tibia may

be partially distributed by the interosseous membrane. This may in turn result in a decrease in the remodelling rate in this region of the bone and therefore an increase in the relative persistence of the epiphyseal scar. The attachment of the inter-osseous membrane to the lateral side of the tibia will also apply a degree of tension to this area of the bone and may therefore act to stimulate bone remodelling in this region. This may mitigate any effect of the interosseous membrane in load distribution. It should also be considered that in addition to applying tension to the lateral surface of the bone, a degree of compressive loading may be applied to the tibial shaft as a result of its movement relative to the fibula.

In addition to the overall level of persistence of the epiphyseal scar in the lateral third of the distal tibia, the high degree of variation in the assignment of RPS values between females and males suggests that, within this region, bone remodelling may be subject to influence from a sex-specific factor or factors. As this region was the only site in which the mean RPS value was greater in females than males, it is suggested that a higher degree of mechanical loading of this region occurs in males than females. This study also suggests that in males, the lateral and central thirds of the distal tibia are exposed to similar levels of mechanical loading.

Within the literature, it is considered that the trajectory of mechanical loading through the ankle joint is dependent on a number of factors, including those which alter the length of the moment arm of the ground reaction force, as this may affect the trajectory of load applied to the tibio-talar joint in the anterior-posterior position. This may occur as a result of variation in the degree of plantarflexion and dorsiflexion in which the ankle is positioned (Braunstein *et al.*, 2010). This may be attributable to the type of footwear commonly worn by an individual (Nigg, 2001; Speksnijder *et al.*, 2005; Barkema *et al.*, 2012). In particular, reference has been made to the potential effect of wearing high heeled shoes on the trajectory of load through the tibiotalar joint (Barkema *et al.*, 2012). For this to influence the level of persistence of the epiphyseal scar in the distal tibia, prolonged wearing of heeled footwear would be required. It is suggested therefore that this is unlikely to be an influencing factor in level of persistence or obliteration of the epiphyseal scar observed in this study.

It is apparent from the results of this study that the persistence of the epiphyseal scar in the distal tibia is influenced by localised factors, resulting in variation in the assignment of RPS between regions of the bone both within and between sexes. Unlike other regions of the skeleton considered in this study however, there does not appear to be a clear pattern that is strongly indicative of the influence of mechanical loading. It could be suggested that the ambiguous pattern observed in the distal tibia is as a result of the cumulative effects of multiple variables including body weight, gait, and muscle mass and commonly worn footwear.

9 Comparison of the Persistence of Epiphyseal Scars in All Skeletal Areas Examined in this Study

This study examined the radiographic persistence of the epiphyseal scar in five anatomical regions including those commonly used in skeletal age estimation. Each of these regions has been examined independently. The radiographs used in this study were obtained from both sexes and included images of both sides of the body, although these were obtained from separate individuals. To examine the overall characteristics of the persistence of the epiphyseal scar and thereby facilitate a critical discussion of the traditional interpretation of this feature in respect of its position in skeletal age estimation, the findings derived from the analysis of each region will be considered collectively.

9.1 Comparison of the intra and inter-observer analyses between skeletal areas

As with all anthropological assessments, it is imperative that the reliability and repeatability of the approach taken are tested. Throughout this study, the intra-observer and inter-observer consistency were assessed and discussed independently. In addition to assessing the reliability of the scoring system, this has facilitated an analysis of the effect of experience on the repeatability of the scoring system. In addition to experience in the application of the scoring system on its reliability, the effect of experience in radiographic interpretation on the consistency of the method could be assessed.

9.1.1 Comparison of intra-observer analyses between skeletal areas

The percentage intra-observer agreements achieved for each sex cohort in each skeletal site are summarised in Table 9.1.

Table 9.1: Summary of percentage intra-observer agreement according to skeletal area

Skeletal Area	Female	Male
Distal Radius	80.00	76.67
Proximal Humerus	86.67	80.00
Distal Femur	80.00	70.00
Proximal Tibia	80.00	83.33
Distal Tibia	66.67	80.00
Mean	**78.68**	**78.00**

As the assessment of images was undertaken over a period of approximately four months, it is possible to assess the effect of increasing experience in the application of the method through the order in which the assessments were conducted. The data presented in Table 9.1 suggest that there was no observable increase in the level of intra-observer agreement through the sample. In each skeletal region, assessment of the images from the female sample was conducted first. It may be reasonable to hypothesise that the experience gained from conducting these assessments may lead to a greater level of intra-observer reliability within the male sample; however this is not supported by the findings of this study. These results indicate that experience in the application of the method does not result in an increase in consistency of the method. The mean percentage intra-observer agreement achieved in both males and females was approximately 78%.

9.1.2 Comparison of inter-observer analyses between skeletal areas

Throughout this study, the level of inter-observer agreement was calculated for each pair of observers within each skeletal area. These analyses have been supplemented by the calculation of the mean percentage agreements between each pair of observers across all skeletal areas and the mean percentage agreement between all observers in each skeletal area. The summarised data are presented in Table 9.2 and Table 9.3 for females and males respectively.

Table 9.2: Summary of the percentage inter-observer agreement in the female sample, the overall mean percentage agreement between observer pairs and the mean inter-observer agreement in all skeletal areas

Skeletal Area	Observer 1 v Observer 2	Observer 1 v Observer 3	Observer 2 v Observer 3	Mean
Distal Radius	86.67	63.33	66.67	**72.22**
Proximal Humerus	80.00	80.00	93.33	**84.44**
Distal Femur	83.33	70.00	90.00	**81.11**
Proximal Tibia	86.67	86.67	100.00	**91.11**
Distal Tibia	90.00	86.67	83.33	**86.67**
Mean	**85.33**	**77.33**	**86.67**	**83.11**

Table 9.3: Summary of the percentage inter-observer agreement in the male sample, the overall mean percentage agreement between observer pairs and the mean inter-observer agreement for each skeletal area

Skeletal Area	Observer 1 v Observer 2	Observer 1 v Observer 3	Observer 2 v Observer 3	Mean
Distal Radius	93.33	83.33	76.67	**84.44**
Proximal Humerus	83.33	83.33	86.67	**84.44**
Distal Femur	53.33	73.33	80.00	**68.89**
Proximal Tibia	66.67	80.00	83.33	**76.67**
Distal Tibia	73.33	83.33	83.33	**80.00**
Mean	**74.00**	**80.66**	**82.00**	**78.89**

The greatest mean percentage agreement was found between observers 2 and 3 in both sex cohorts. This pair of observers represented the highest levels of experience in skeletal age estimation and radiographic interpretation. The lowest mean inter-observer comparison was found in the pairing of observers 1 and 3 in the female sample and 1 and 2 in the male sample. In contrast to the pairings in which the greatest inter-observer agreements were observed, those exhibiting the lowest agreement involved the individual with the least experience in radiographic image interpretation or skeletal age assessment. As the lowest percentage agreements were found to involve the novice observer in both sex samples and the highest percentage agreements occurred between the individuals of greatest experiential level, it is suggested that experience in radiographic interpretation may be of greater benefit to the consistent application of the scoring system.

9.2 Comparison of the total persistence rate between skeletal areas

The critical discussion of the persistence of the epiphyseal scar must begin with hypothesis that this feature will, over time, obliterate. The TPR of the epiphyseal scar in each skeletal region examined in this study according to sex is summarised in Table 9.4. These data show that in females, the highest persistence of the epiphyseal scar was observed in the distal femur, while in males, this was observed in the proximal tibia. The lowest persistence rates in both sex cohorts were observed in the distal radius. These data also suggest however that the persistence of the epiphyseal scar within the upper and lower limbs may decrease in a proximal-distal direction, with those regions closest to the trunk exhibiting higher persistence rates than observed in the more distal areas.

Table 9.4: Total persistence rate according to sex and skeletal area

Skeletal Area	Female TPR	Male TPR
Distal Radius	86.04	77.92
Proximal Humerus	94.19	94.82
Distal Femur	99.26	97.23*
Proximal Tibia	98.05	97.74*
Distal Tibia	92.72	92.95*

*Difference between males and females was statistically significant ($p<0.05$)

As the analysis of the distal femur was undertaken in an alternative radiographic plane, the data from this skeletal region may not be directly comparable with those analyses conducted on radiographs taken in the A-P plane. Therefore, although in the male sample, a small increase was observed in the TPR between the distal femur and proximal tibia, it may not be appropriate to directly compare the values obtained.

To ascertain the statistical significance of the variation in persistence of the epiphyseal scar between the five skeletal regions, a series of one-way ANOVA was conducted. While a statistically significant degree of variation in the assignment of TPS between regions was observed in both the female ($P<0.001$) and male ($P<0.001$), the application of Dunns pairwise comparison procedures showed that no statistically significant difference was observed in the assignment of TPS between the proximal and distal tibia; and the proximal humerus and distal radius in either sex cohort. In addition, within the female sample, no statistically significant difference was observed in the TPS values assigned to the distal femur and distal tibia.

No statistically significant difference was observed between the left and right sides of the body in either the female ($P=0.407$) or male ($P=0.250$) complete samples; however analysis of the variation observed in the persistence of the epiphyseal scar between left and right sides of the body in each skeletal area suggested evidence of a pattern. The results of these analyses are summarised in Table 9.5.

Table 9.5: Summary of the statistical significance of variation in the persistence of the epiphyseal scar between left and right sides of the body according to biological sex and skeletal area

Skeletal Area	Female	Male
Distal Radius	0.288	0.536
Proximal Humerus	0.653	0.762
Distal Femur	0.001	<0.001
Proximal Tibia	<0.001	<0.001
Distal Tibia	0.144	<0.001

As the primary factors assessed during this study, the influences of chronological age, biological sex and side of the body on the persistence of the epiphyseal scar were analysed through the application of GLM analyses. The results of these analyses in respect of these factors are summarised in Table 9.6.

Table 9.6: Summary of the adjusted coefficients of determination (R^2) of the relationships between Total Persistence Score and chronological age, biological sex and side of the body according to skeletal area

Skeletal Area	Age	Sex	Side
Distal Radius	0.011	0.004	-0.001
Proximal Humerus	0.025*	0.002	-0.001
Distal Femur	-0.025	0.078*	-0.001
Proximal Tibia	0.027*	0.045*	0.036*
Distal Tibia	0.076*	0.009*	0.002

*Statistically significant ($p<0.05$)

These data suggest that statistically significant interactions between all three factors (chronological age, biological sex and side of the body) and the TPS only occurred in the proximal tibia. Of these variables, biological sex was observed to exhibit the strongest relationship with TPS; however this explained less than 5% of the variation in the persistence of the epiphyseal scar within this region. Within the distal tibia, only the interaction between side of the body and TPS was not found to be statistically significant. In this region, the chronological age was found to exert the strongest effect on the persistence of the epiphyseal scar. The coefficient of determination achieved for this interaction suggested that 7.6% of the variation in the persistence of the epiphyseal scar was attributable to variation in chronological age.

At the opposing end of the spectrum, in the distal radius, no statistically significant relationships were observed between chronological age, biological sex or side of

the body and persistence of the epiphyseal scar. This indicates that persistence of the epiphyseal scar within this region must be influenced by factors other than those included in this study. In a similar pattern, within the proximal humerus, no statistically significant relationship was observed between the persistence of the epiphyseal scar and biological sex or side of the body. The interaction between chronological age and TPS was found to be statistically significant; however this explained only 2.5% of the variation in the persistence of the feature in this anatomical region.

These analyses suggest that the chronological age, biological sex and side of the body exert a greater level of influence on the persistence of the epiphyseal scar within the lower limb than is observed in the upper limb. This suggests that the variation between the upper limb and lower limb may be related to a factor or factors which combine the effects of these three variables. Further GLM analyses were undertaken to ascertain the combined influence of these variables on the persistence of the epiphyseal scar in each of the five regions. These analyses supported the initial observation that the level of persistence of the distal radial epiphyseal scar is largely uninfluenced by the factors examined in this study. Similarly, no further statistically significant interactions were observed between the persistence of the epiphyseal scar in the proximal humerus and chronological age, biological sex or side of the body.

Within the distal femur, the combined effects of all three regions exhibited the strongest statistically significant model for the variation in the persistence of the epiphyseal scar in the proximal humerus and may account for 20.4% of the variation in the persistence of the feature in this skeletal area. In the proximal tibia, it was observed that the interaction between age and sex exhibited the strongest relationship with TPS. This interaction explained 10.1% of the variation in the epiphyseal scar in this region. Within the distal tibia, the highest coefficient of determination was observed in the interaction between biological sex and side of the body and TPS. This relationship explained 27% of the variation in the persistence of the epiphyseal scar within this region. Overall, these analyses indicate that while the variables examined in this study may influence the persistence of the epiphyseal scar to a certain degree, the strength of the

relationship between these variables and the assignment of TPS values does not appear to be of sufficient strength to support a causative link between these factors and the level of persistence or conversely, level of obliteration, of the epiphyseal scar.

To examine the persistence or obliteration of the epiphyseal scar in greater detail, and thereby facilitate a more in-depth analysis of any patterns in the persistence of the feature, discrete regional analyses were conducted. Due to the orientation in which the radiographs of the distal femur were assessed, this region will be discussed in relation to the pattern of persistence rather than the observed level of persistence.

Summaries of the mean RPS values assigned to the medial, central and lateral regions of each skeletal area are summarised in Table 9.7 and Table 9.8 for females and males respectively.

Table 9.7: Summary of the mean Regional Persistence Scores in female individuals according to skeletal area

Skeletal Area	Medial	Central	Lateral
Distal Radius	1.08	1.41	1.17
Proximal Humerus	0.75	1.74	1.16
Proximal Tibia	2.37	2.05	1.64
Distal Tibia	1.20	1.97	2.73

Table 9.8: Summary of the mean Regional Persistence Scores in male individuals according to skeletal area

Skeletal Area	Medial	Central	Lateral
Distal Radius	1.09	1.26	0.95
Proximal Humerus	0.76	1.84	0.89
Proximal Tibia	2.02	1.63	1.41
Distal Tibia	1.37	2.04	1.86

These data show that within the upper limb, a similar pattern occurred in the maximum and minimum mean RPS values in both sex cohorts. In both females and males, the maximum mean RPS values for the upper limb were observed in the central third of the respective bones. Within the distal radius, the lowest mean RPS values were observed in the medial and lateral thirds of the bone for females and males respectively, while in the proximal humerus, this was found in the medial third of the bone in both sex cohorts. As these data suggested a degree of

similarity in the persistence of the epiphyseal scar within the lateral third of the distal radius and proximal humerus in both sexes, a series of one-way ANOVA were conducted to assess the degree of variation between the data attributed to these regions. The results of these analyses suggested that there was no statistically significant variation between the persistence of the epiphyseal scar within the lateral third of the radius and humerus in either females (P=0.422) or males (P=0.955). This indicates that remodelling of the epiphyseal scar within the lateral thirds of the distal radius and proximal humerus is subject to similar degrees of influence.

Within the lower limb, the maximum persistence in the proximal tibia was observed in the medial third of the bone; while the minimum mean RPS values were occurred in the lateral third in both sex cohorts. The pattern of maxima and minima found in the distal tibia differed to that observed in the proximal tibia. In both females and males, the minimum mean RPS value was detected in the medial third of the bone. The location of the maximum mean RPS value however differed between sexes, occurring in the lateral third of the bone in females and the central third of the bone in males.

The statistical significance of the variation in RPS between females and males was calculated through a series of one-way ANOVA. The results of these analyses (summarised in Table 9.9) suggest that a greater degree of inter-sex variation in the regional persistence of the epiphyseal scar may occur in the lower limb than the upper limb.

Table 9.9: Statistical significance of the inter-sex variation between Regional Persistence Scores in each region according to skeletal area

	Medial	Central	Lateral
Distal Radius	0.445	0.962	0.019
Proximal Humerus	0.660	0.071	<0.001
Proximal Tibia	<0.001	<0.001	0.013
Distal Tibia	0.001	0.464	<0.001

The proximal tibia was the only skeletal area in which statistically significant levels of inter-sex variation were observed in each of the three regions of the bone. This may suggest that persistence of the epiphyseal scar in this region is under greater

influence from sex-related factors than other areas of the skeleton. Similarly, of the skeletal areas included in this study, the lateral third was the only region in which statistically significant levels of inter-sex variation were observed in all sites. This may suggest that remodelling within this region is most susceptible to influence from sex-related factors.

Further analyses of the variation in the persistence of the epiphyseal scar between the medial, central and lateral regions of the respective bones were conducted at each skeletal site. The results of these studies, which are summarised in Table 9.10 and Table 9.11 for females and males respectively, suggest that the variation observed between regions of the upper limb may be less statistically significant than that found in the lower limb in both females and males.

Table 9.10: Summary of the statistical significance of the variation in the persistence of the epiphyseal scar between regions of the bone in each skeletal area in female individuals

	Medial v Central	Central v Lateral	Lateral v Medial
Distal Radius	<0.001	0.012	0.201
Proximal Humerus	<0.001	<0.001	<0.001
Proximal Tibia	<0.001	<0.001	<0.001
Distal Tibia	<0.001	<0.001	<0.001

Table 9.11: Summary of the statistical significance of the variation in persistence of the epiphyseal scar between regions of the bone in each skeletal area in male individuals

	Medial v Central	Central v Lateral	Lateral v Medial
Distal Radius	0.043	<0.001	0.081
Proximal Humerus	<0.001	<0.001	0.022
Proximal Tibia	<0.001	0.018	<0.001
Distal Tibia	<0.001	0.105	<0.001

A similar pattern in the distribution of statistically significant results was observed in females and males, where it was observed that only the variation between the lateral and medial thirds of the distal radius were not statistically significant in either sex. Additionally, in the male sample, no statistically significant difference was observed in the interaction between the central and lateral regions of the distal tibia. Based on the results of these findings, it is suggested that females may exhibit a greater degree of variation in the persistence of the epiphyseal scar than males at these skeletal sites.

The regional intrabone analyses suggest that statistically significant variation in the persistence of the epiphyseal scar may exist within skeletal regions and therefore indicates that localised rather than systemic factors may influence the persistence of the epiphyseal scar within skeletal regions. In addition, the degree to which these factors influences the persistence of the epiphyseal scar appears to be variable between anatomical sites, as one might expect if there are localised influences.

Table 9.12: Results of general linear models for the regional persistence of the epiphyseal scar in all skeletal areas (excluding the distal femur)

Factor(s)	Significance	R^2	Adjusted R^2
Area	<0.001	0.09	0.089
Region	<0.001	0.022	0.022
Side	0.106	0.000	0.000
Sex	<0.001	0.006	0.006
CA	<0.001	0.022	0.018
Area * Region	<0.001	0.179	0.177
Area * Side	<0.001	0.095	0.094
Area * Sex	0.001	0.098	0.097
Area * CA	<0.001	0.138	0.123
Region * Side	0.738	0.023	0.022
Region * Sex	<0.001	0.033	0.032
Region * CA	0.922	0.05	0.038
Side * Sex	0.028	0.007	0.007
Side * CA	0.132	0.027	0.019
Sex * CA	<0.001	0.037	0.029
Area * Region * Side	0.384	0.185	0.182
Area * Region * Sex	<0.001	0.199	0.196
Area * Region * CA	0.689	0.251	0.211
Area * Side * Sex	<0.001	0.107	0.105
Area * Side * CA	<0.001	0.168	0.139
Area * Sex * CA	<0.001	0.177	0.149
Region * Side * Sex	0.701	0.034	0.033
Region * Side * CA	0.999	0.059	0.035
Region * Sex * CA	0.998	0.074	0.050
Side * Sex * CA	0.002	0.051	0.035
Area * Region * Side * Sex	0.048	0.210	0.205
Area * Region * Side * CA	1.000	0.298	0.219
Area * Region * Sex * CA	0.998	0.32	0.244
Area * Side * Sex * CA	0.009	0.232	0.177
Region * Side * Sex * CA	0.999	0.097	0.049
Area * Region * Side * Sex * CA	0.994	0.411	0.262

A final series of GLM analyses were undertaken to ascertain the variation in the regional persistence of the epiphyseal scar which was attributable to the region of

the bone, the skeletal area, sex, chronological age or side of the body on which the assessment was undertaken. The results of these analyses, summarised in Table 9.12, suggest that although chronological age and biological sex exhibit statistically significant relationships with the regional persistence of the epiphyseal scar, the greatest variation attributable to a single factor is related to the location of the feature within the skeleton. This factor was found to explain 8.9% of the variation in the regional persistence of the epiphyseal scar. Following this, the region of the epiphyseal scar was observed to account for 2.2% of the variation in the epiphyseal scar.

Subsequent GLM analyses showed that the percentage of the variation in the persistence of the epiphyseal scar attributable to the combined variation in skeletal area and region of the bone was 17.7%. When the variation attributable to the effects of biological sex was included in the analysis, the coefficient of determination of the model increased to 19.6%. The strongest general linear model however was observed to occur within the interaction of skeletal area, region of the bone, side of the body and biological sex. This interaction, although only marginally statistically significant, was found to account for 20.5% of the variation in the regional persistence of the epiphyseal scar.

These findings indicate that the regional variation in the persistence of the scar is not significantly influenced by the chronological age of the individual. Based on the results of this study, it is also suggested that the variation observed in the persistence of the epiphyseal scar is most likely attributable to factors related to the location of the epiphyseal scar within the skeleton and the localised factors to which each region is exposed. Influences related to the biological sex of the individual may enhance the effect of the localised causes of bone remodelling, however the relative weakness of the observed relationship between biological sex and the regional persistence of the epiphyseal scar suggests that this element does not exert a strong independent effect on the persistence or obliteration of this feature.

The results of a series of GLM analyses conducted on the data derived from the assessment of the distal femur showed that region of the bone and sex of the

individual exhibited a statistically significant relationship with TPS and explained 26.2% of the variation in the persistence of the epiphyseal scar. This finding supports those pertaining to the remainder of the skeletal areas included in this study which suggested that remodelling of the epiphyseal scar occurs partially as an effect of localised factors.

10 General Discussion

The persistence of the epiphyseal scar in adult individuals has been a matter of debate for almost a century, with some researchers asserting that the feature is associated with recent fusion and thereby inferring that it will obliterate through the continuous process of bone remodelling; however other researchers have acknowledged the potential persistence of this feature in adult individuals in some anatomical regions (Todd, 1937; Garden, 1961; Hoerr *et al.*, 1962; Hall and Rosser, 1963; MacLaughlin, 1987; Schmeling *et al.*, 2004; Schulz *et al.*, 2005; Schulz *et al.*, 2008a; Baumann *et al.*, 2009; Kellinghaus *et al.*, 2010). Consequently, the observation of an epiphyseal scar on a radiographic image has been taken as an indication of recent fusion and by extension the absence of an epiphyseal scar has been linked with individuals of older chronological age; however no clear evidence has been presented in the literature that validates this position. This study was undertaken with the aim of establishing the relationship between the persistence of the epiphyseal scar and factors including chronological age, biological sex and side of the body in five anatomical regions in both the upper and lower limbs.

10.1 The data

This study was, by necessity, conducted using a sample of radiographic images. Anterior-posterior and lateral radiographs of five anatomical areas were collected and examined to assess the level of persistence of the epiphyseal scar. Due to the legal and ethical restrictions that surround the use of ionising radiation for non-therapeutic purposes, it is not possible to replicate the longitudinal studies of growth, maturation and development conducted in North America and Europe during the first half of the 20th century (Hoerr *et al.*, 1962; Pyle and Hoerr, 1969; Garn, 1981; DEFRA, 2004; Schmeling *et al.*, 2007; Hackman and Black, 2013b). As a result, it was necessary to undertake this study using a cross-sectional sample of radiographic images obtained from clinical sources. Although it has been acknowledged that this methodological approach is most suited to the calculation of prevalence, the resulting data may be explained by numerous factors, thus introducing the problem of associating correlated and causative effects with any observed pattern or trend (Mann, 2003). In addition, while it is assumed that the

variation observed between age cohorts in a cross-sectional sample is illustrative of the variation within the life-span of a single individual, this may not be accurate in all cases (Borkan *et al.*, 1983).

As the radiographs utilised in this study were obtained from a clinical sample, several limitations on the study were unavoidable. This included the effects of the orientation of the image and the effects of superimposition of structures. As the original purpose of the images was to obtain the optimal view of a suspected trauma or pathology, the orientation of the radiographs included in this study was not constant within or between anatomical regions. This necessitated the development of a method that utilised anatomical landmarks as the grounding points for the assessment grid.

A radiographic image is a two-dimensional representation of a three-dimensional structure, it is necessary to consider the influence that superimposition of structures may have had on the interpretation of the epiphyseal scar (Cotti and Campisi, 2004; Jennane *et al.*, 2007). The effects of superimposition of overlying structures were found to be particularly problematic in the region of the distal femur where, due to the location of the patella in the anterior-posterior plane, the region of the epiphyseal scar was obstructed from view. This resulted in all assessments of the distal femur being undertaken in the medial-lateral plane. Although this provided an unobstructed view of the region of the epiphyseal scar, the variation in angle of observation between this and the remaining anatomical regions reduced the level of comparison that could be made between this and other skeletal areas.

In addition to the effects of overlying osseous structures, radiographic superimposition should be considered during the interpretation of epiphyseal scars within the discrete regions of the bone. As an area of greater bone density is reflected in the radiographic image as a line of increased relative radio-opacity, areas of the skeleton of a greater diameter may appear to exhibit a greater level of persistence of the epiphyseal scar as the beam must pass through a greater quantity of bone than the surrounding area.

10.2 Discussion of research findings

10.2.1 Reliability of assessments of the epiphyseal scar in radiographs from adult individuals

The effect of training on the reliability of methods of skeletal assessment has been thoroughly documented, particularly in relation to those methods of skeletal age assessment commonly used in forensic practice (Roche *et al.*, 1970; Lynnerup *et al.*, 2008; Rajan *et al.*, 2011). The term "reliability" may be considered to include both repeatability of assessments when conducted by a single observer and the comparability between assessments carried out by multiple observers (Johnson *et al.*, 1973). In forensic practice, it is not sufficient for a method of assessment to be accurate; it must also satisfy the criteria of reliability (The Law Commission, 2011).

This study examined intra-observer and inter-observer through the assessment of a subsample of 30 left side radiographs for each sex in each anatomical area. Intra-observer results of this study yielded a mean percentage intra-observer agreement of approximately 78% in both sex cohorts. Although no published literature exists with which to compare the levels of intra-observer agreement found in this study, a high degree of intra-observer error has been noted in the examination and rating of other radio-opaque lines (MacChiarelli *et al.*, 1994).

No overall pattern was observed in the percentage intra-observer agreement obtained within anatomical regions in relation to the order in which assessments were conducted. This finding was not expected as training or experience in the application of a method of skeletal assessment is generally considered to be beneficial to intra-observer consistency, (Rajan *et al.*, 2011). It was noted however that the lowest percentage agreement across all anatomical areas and both sexes, observed in the female distal tibia, corresponded to the data set on which the first assessments were conducted. This finding may indicate that some training or experience in the application of the method is required initially, after which the intra-observer agreement reaches a plateau that is unaffected by further experience or training.

The comparability of assessments between multiple observers is fundamental to skeletal age estimation methods and is as important as intra-observer

repeatability. Inter-observer assessments were conducted in all skeletal areas by three observers who represented varying levels of experience in radiographic interpretation. These results of these analyses showed that the mean percentage agreement between pairs of observers ranged between 74 - 82% in males and 77.33 - 86.67% in females. These results are consistent with those achieved in the intra-observer analyses and therefore may support the proposition that experience in the application of the method may not exert a significant effect on the repeatability or comparability of the assessment. Comparison of the data achieved from the inter-observer analysis of the epiphyseal scar with those obtained from the inter-observer assessment of other radio-opaque and radiolucent lines suggests that the percentage of inter-observer agreement achieved in this study exceeded that observed in other inter-observer assessments (MacChiarelli *et al.*, 1994; Kneif *et al.*, 2005).

In addition to the overall percentage agreement, the calculation of the mean percentage agreement between observers facilitated an analysis of the effect of experience in radiographic interpretation on the application of the method for assessing the persistence of epiphyseal scars. In both sex cohorts, the greatest mean percentage agreement was achieved between the observers with the highest level of experience in the interpretation of radiographs (observers 2 and 3). Similarly, the lowest mean percentage agreement involved the observers with the least (observer 1) and most (observer 3) experience in radiographic interpretation. These findings indicate that the experience in reading radiographic images may confer a beneficial effect on the comparability of the results between observers where individuals of a similar level of experience are more likely to return comparable results. This pattern was to be expected given the reported effects of training and experience on inter-observer agreement (Roche *et al.*, 1970; Rajan *et al.*, 2011). As the experience of the author in radiographic interpretation is considered to lie between that of observers 1 and 2, the results of the intra-observer analysis are consistent with the hypothesis that experience in radiographic interpretation is a determining factor in the overall reliability of assessments of the level of persistence of epiphyseal scars in adult individuals.

10.2.2 Overall trends in the persistence of epiphyseal scars

Persistent epiphyseal scars were noted in the majority of individuals in all anatomical areas included in this study. This finding seemingly contradicts much of the published literature relating to the persistence of epiphyseal scars in the long bones of adult individuals, where it is generally considered that the feature will likely obliterate soon after the completion of epiphyseal fusion (Greulich and Pyle, 1959; Garden, 1961; Workshop of European Anthropologists, 1980; Whitaker *et al.*, 2002). Subsequent observations regarding the trend in TPR between the anatomical regions considered by this study suggested that the level of persistence of the epiphyseal scar may decrease in a proximal-distal direction in both the upper and lower limbs, when viewed in the anterior-posterior plane. Although there is a paucity of published research relating to the persistence of the epiphyseal scar in adult individuals, the trend observed in this study may be consistent with the findings of Weiss *et al*. (2012) who, within the age ranges included in this study, noted the persistence of epiphyseal scars in 46.67% of the first metatarsals examined.

As this study was, by necessity, based on a cross-sectional radiographic sample, only information relating to the chronological age, biological sex and side of the body from which the image was obtained were available. Consequently, the following sections will discuss the overall trends observed in the persistence of the epiphyseal scar in relation to these factors.

10.2.2.1 The persistence of epiphyseal scars in relation to chronological age

The relationship between chronological age and the observation of the epiphyseal scar is one that has divided opinion within the literature. While some early studies did not exclude the possibility of the persistence of the epiphyseal scar in the long bones of adult individuals, these have largely been replaced by those in which the obliteration of the epiphyseal scar is included as a maturity criterion (Cope, 1920; Hoerr *et al.*, 1962; Pyle and Hoerr, 1969; Acsadi and Nemeskeri, 1970; Workshop of European Anthropologists, 1980; O'Connor *et al.*, 2008; Schmidt *et al.*, 2008; Baumann *et al.*, 2009; Cameriere *et al.*, 2012). Variation in the level of persistence of the epiphyseal scar undoubtedly exists between skeletal elements, as shown by

the result of this study; despite this, there appears to be a willingness to accept that the pattern of obliteration of the feature observed in one anatomical region may be transposed to another without adequate testing (Schmidt *et al.*, 2008; Baumann *et al.*, 2009).

As the primary characteristic with which the obliteration of the epiphyseal scar is associated, assessment of the relationship between chronological age and TPS was undertaken. In contrast to the majority of published sources, the epiphyseal scars of all the regions considered in this study exhibited the potential to persist in to the fifth decade of life. The results of this study showed some similarity with the discord present within the literature, as these analyses indicated that the strength and significance of the relationship between chronological age and the level of persistence of the epiphyseal scar was not constant between skeletal elements. This suggests that, in addition to systemic factors, localised influences may affect the persistence of the epiphyseal scar.

While the results of this study appear to disagree with the premise on which several methods of radiographic skeletal age estimation are based, it appears to support the findings of Acsadi and Nemeskeri (1970), who in their "complex method" determined that the epiphyseal scar of the proximal humerus was likely to remain, despite loss of the surrounding trabecular bone. This study also supports the statement made by Baumann *et al.* (2009) in relation to the potential persistence of the epiphyseal scar in the distal radius beyond 30 years of age.

Although it is clear that the persistence or obliteration of the epiphyseal scars of the regions considered in this study occurs predominantly independently of the chronological age of the individual, a close relationship between chronological age and the disappearance of the epiphyseal scar in some areas has facilitated the development of an approach to skeletal age estimation which is common to many groups (Kreitner *et al.*, 1998; Schulz *et al.*, 2005; Schulz *et al.*, 2008a; Garamendi *et al.*, 2011; Gonsior *et al.*, 2013). This strengthens the hypothesis that localised factors in addition to systemic influences affect the level of persistence of the epiphyseal scar and the rate at which it may obliterate. It is clear, however, that factors other than chronological age are responsible for the majority of variation in

the level of persistence of the epiphyseal scar observed in this cross-section of the sample population.

10.2.2.2 The persistence of epiphyseal scars in relation to biological sex

The variation in the timing of skeletal development and epiphyseal fusion has been thoroughly documented (Flory, 1935; Hansman and Maresh, 1961; Garn *et al.*, 1974; Lampl and Jeanty, 2003). As the epiphyseal scar is formed as a result of the completion of epiphyseal fusion and, it has been suggested, may remodel within the following two years, it would be reasonable to expect a pattern in the inter-sex variation in the level of persistence of the epiphyseal scar (Greulich and Pyle, 1959; Workshop of European Anthropologists, 1980). This pattern may be expected to follow that of epiphyseal fusion, where the epiphyseal scar of those sites with the earliest fusion age is observed to undergo the greatest remodelling.

Many of the effects of biological sex on the skeleton derive from the action of systemic hormones including oestrogens and androgens on bone remodelling processes throughout the skeleton (Compston, 2001; Notelovitz, 2002; Balasch, 2003). If inter-sex variation in the persistence of the epiphyseal scar was solely attributable to differences in the levels of circulating hormones, the level of obliteration would be similar throughout the skeleton. Contrary to the proposed hypothesis, this study observed that variation in the overall persistence of the epiphyseal scar between females and males was only statistically significant within the bones of the lower limb. This indicates that the level of persistence or obliteration of the epiphyseal scar may be partially related to localised sex-related factors, for example variation in the localised mechanical loading applied by skeletal muscle or total body mass, in addition to systemic influences (Frontera *et al.*, 1991; Gallagher *et al.*, 1997; Compston, 2001; Notelovitz, 2002; Abe *et al.*, 2003; Balasch, 2003; Wells, 2007).

Within the lower limb, the strength of the relationship between biological sex and TPS, as defined by the coefficient of determination, was found to decrease in a proximal-distal direction, indicating that the influence of sex-related factors on the persistence of the epiphyseal scar may decrease in a distal direction. Although statistically significant, the maximum variation in the assignment of TPS and

therefore the persistence of the epiphyseal scar was less than 8%, indicating that the majority of variation in the persistence of the feature is not attributable to systemic factors related to biological sex.

10.2.2.3 The persistence of epiphyseal scars in relation to side of the body

Bilateral asymmetry in skeletal morphology as a result of functional limb dominance has been widely reported and in some studies has been linked to an increased muscle mass in the dominant limb compared with the non-dominant limb (Ruff and Jones, 1981; Trinkaus *et al.*, 1994; Sadeghi *et al.*, 2000; Auerbach and Ruff, 2006; Kanchan, 2008; Lazenby *et al.*, 2008; Blackburn, 2011). Although bilateral asymmetry has been reported in the upper limb, particularly in the humerus (Blackburn, 2011; Ozener, 2012); it is considered to occur less frequently in the lower limb, perhaps as a result of an increased homogeneity of function in the weight bearing lower limbs than observed in the upper limb, where functional dominance may be more pronounced (Cuk *et al.*, 2001; Plochocki, 2004; Auerbach and Ruff, 2006; Kanchan, 2008). It was therefore reasonable to suggest that a statistically significant degree of variation may exist in the persistence of epiphyseal scars in the left and right sides of the body.

Contrary to the published literature which suggests that bilateral asymmetry in skeletal morphology is generally more pronounced in the upper limb, the results of this study indicate that bilateral asymmetry in the persistence of the epiphyseal scar is more evident in the lower limb than the upper limb (Ruff and Jones, 1981; Auerbach and Ruff, 2006). With the exception of the female distal tibia, the variation in the persistence of the epiphyseal scars of the lower limb between the left and right sides of the body was statistically significant. This contrasts with the upper limb where no statistically significant variation was observed. As the majority of studies concerning bilateral asymmetry are based on an analysis of cortical bone and external gross osseous morphology, the discrepancy between the hypothesised result and that obtained from this study could be attributable to a differential response of trabecular and cortical bone to asymmetries in mechanical loading (Ruff and Jones, 1981; Haapasalo *et al.*, 1996; Ozener, 2012).

As this analysis was undertaken on a cross-section of the population from a clinical source, it was not possible to assess the persistence of the epiphyseal scar in relation to known limb dominance. There is no reason, however, to expect the study population to deviate from the population norm where, it is reported, approximately 90% of individuals express right-sided dominance in the upper limb, while 55-75% of individuals reportedly express left-sided dominance in the lower limb (Cuk *et al.*, 2001; Blackburn, 2011). The absence of a statistically significant degree of variation in the persistence of the epiphyseal scar in the proximal humerus or distal radius suggests that limb dominance may not alter the observed level of persistence or obliteration of the epiphyseal scar in the upper limb.

10.2.2.4 The persistence of the epiphyseal scar in relation to the combined effects of chronological age, biological sex and side of the body

Based on the initial findings of this study, it is suggested that although biological sex, chronological age and functional dominance may, to varying degrees, exert an effect on the persistence or obliteration of the epiphyseal scar, their interaction with bone remodelling is dependent on additional factors. While the initial analyses conducted in this study suggest that biological sex, chronological age and side of the body exhibit weak relationships with the persistence of epiphyseal scars; these factors exhibit a large degree of cross-over in their potential interactions with the musculoskeletal system (Lindle *et al.*, 1997; Lee *et al.*, 2000; Doherty, 2001; Abe *et al.*, 2003; Doherty, 2003; Ditroilo *et al.*, 2010). The interactions between the effects of chronological age, biological sex and/or side of the body were supported by the findings of this study which found that in all regions of the lower limb, paired or complex interactions explained the greatest proportion of variation in the persistence of the epiphyseal scar.

Although the effects of some factors related to chronological age or biological sex may be systemic, the observed discrepancies in the persistence of the epiphyseal scar within and between the upper and lower limbs in both sexes suggests that localised influences may play a significant role in the observed level of persistence or obliteration of the feature in adult individuals. This may be related to the site-

specific nature of bone remodelling (Crowder and Austin, 2005; Modlesky *et al.*, 2011; Turunen *et al.*, 2013).

10.2.3 Regional trends in the persistence of epiphyseal scars

To assess the potential role of localised remodelling in the persistence or obliteration of the epiphyseal scar, analyses of the variation in RPS values within discrete regions of the epiphyseal scar were undertaken for each anatomical site. Due to the potential for the patella to obscure the distal femoral epiphyseal scar in anterior-posterior view radiographs, this area was assessed in the medial-lateral plane. Consequently, only the trends in persistence or obliteration of the epiphyseal scar in this site may be compared with the remaining skeletal regions.

Initial analysis of the regional persistence of the epiphyseal scar was undertaken in each skeletal area through the calculation of the mean RPS value for each of the medial, central and lateral thirds of the bone in the proximal and distal tibial, proximal humeral and distal radial epiphyseal scars; and in the anterior, central and posterior third of the distal femoral epiphyseal scar. Although obtained from multiple skeletal sites, the resulting data suggested that the persistence or obliteration of the epiphyseal scar is likely to vary both within and between anatomical areas in both female and male individuals.

Analysis of assigned RPS values between the discrete regions in specific skeletal areas indicated that statistically significant levels of inter-sex variation in the persistence of the epiphyseal scar were more likely to occur in the lower limb than the upper limb. This observation is supported by the results of the analysis of the distal femur which indicated that a statistically significant inter-sex difference existed in the persistence of the epiphyseal scar in the anterior, central and posterior regions of the bone.

Further analyses showed that, with the exception of the lateral and medial thirds of the distal radius in both sexes; and the central and lateral thirds of the distal tibia in males, statistically significant degrees of variation in the persistence of the epiphyseal scar occurred between discrete regions within individual skeletal areas. Statistically significant degrees of variation were also observed in the persistence of the epiphyseal scar between all regions of the distal femur in both sexes. These

findings support the proposition that the persistence or obliteration of the epiphyseal scar is likely to be influenced by both systemic and localised factors which vary within and between skeletal areas.

As an extension of the initial analyses conducted during this phase of the study, the relationships between biological sex, chronological age and side of the body and the regional persistence of epiphyseal scars were assessed. The results of these analyses suggested that in three of the five skeletal areas considered in this study, the strongest statistically significant relationship with RPS values occurred between region of the bone and the biological sex of the individual. Models based on these factors explained between 13.6% and 26.2% of the variation in the regional persistence of the epiphyseal scar. In addition to this interaction, in the proximal tibia, the strongest statistically significant relationship was observed between region of the bone, biological sex and side of the body; however this model explained only 10.5% of the variation in RPS. The distal radius was the only skeletal area in which region of the bone was not included in the strongest statistical model. At this site, the combined effects of chronological age and biological sex were found to exhibit the strongest relationship with RPS; however this model explained less than 6% of the variation in the regional persistence of the epiphyseal scar.

A final series of GLM analyses were conducted to assess the relationships between RPS values and all potential explanatory variables (biological sex, chronological age, side of the body, region of the bone and skeletal area). The results of these analyses suggested that the interaction between skeletal area, region of the bone (medial, central or lateral) and biological sex was the strongest, statistically significant model for explaining the variation in RPS. These results further support the proposition that the persistence or obliteration of the epiphyseal scar may be influenced additionally by the skeletal location and localised factors which affect the persistence of the epiphyseal scar in discrete regions of the bone (Modlesky *et al.*, 2011; Turunen *et al.*, 2013).

Based on these findings, it is suggested that the variation in the persistence of the epiphyseal scar observed within and between skeletal areas may be partially

attributable to the degree and trajectory of mechanical loading to which the bone is exposed.

10.2.4 The persistence of the epiphyseal scar: A new paradigm

Since the work of Julius Wolff, in which it was postulated that the cancellous structure of a long bone would remodel to align along the principal force trajectory while minimising the mass of the bone, the potential role of mechanical loading in bone remodelling has been a matter of discussion in the literature related to bone biomechanics (Roesler, 1987; Turner, 1992; Harrigan and Hamilton, 1994; Lee and Taylor, 1999; Ruff *et al.*, 2006). Although the mathematical principles on which Wolff based his theory has been found to be incompatible with more contemporary research, the basis of bone functional adaptation has been generally acknowledged (Ruff *et al.*, 2006).

Dynamic mechanical loading has been widely accepted as a stimulator of bone remodelling. The degree to which mechanical loading affects bone modelling and remodelling however is believed to be modulated by a genetically pre-determined set point, termed the "mechanostat" (Frost, 1987; 1998b; 2003; Schoenau and Fricke, 2008). Through the stimulation of bone remodelling, the cancellous structure is altered to give rise to volumetric changes which reflect the structural demands to which each bone is subjected (Harrigan and Hamilton, 1994; Bagge, 2000). It has been reported that the mechanical properties of cancellous bone vary between anatomical locations as a result of their function and so, it is therefore reasonable to hypothesise that the rate of bone remodelling which occurs as a result of mechanical loading will also vary between skeletal elements (Goldstein, 1987). This may, in turn, affect the persistence or obliteration of the epiphyseal scar, as shown by the findings of this study.

It has been shown through experimental studies that bone exhibits a higher modulus of elasticity under compression than tension and that this varies between skeletal sites (Carter *et al.*, 1980; Morgan and Keaveny, 2001). This may be the result of an adaptation to the directionality of loads to which the skeleton is commonly exposed as a result of a bipedal stance. As a result of the adaptation of human bone to compressive loading, it is reasonable to suggest that the rate of

remodelling in areas under tension will be greater than that in skeletal areas predominantly under compression. The proposed relationship between mechanical loading and the persistence or obliteration of the epiphyseal scar is supported by the skeletal areas in which epiphyseal scars have been observed, and perhaps more conspicuously, those in which obliteration of the feature has been reported. Throughout the analyses conducted in the formative stages of this study and reported in Chapter 3, no epiphyseal scar was observed at the calcaneal epiphysis or the proximal epiphysis of the fifth metatarsal. At these sites, the sural tendon and the tendon of peroneus brevis muscle respectively create dynamic mechanical loading of the bone to which they attach, resulting in an apophyseal site (Standring, 2008). This obliteration is replicated at other apophyseal sites, including the iliac crest, medial and lateral epicondyles of the humerus and olecranon process where no epiphyseal scars have been reported (Brodeur *et al.*, 1981). This hypothesis is supported by observations made by Parsons (1904) in his formative study of traction epiphyses where the absence of an epiphyseal line was noted in the greater trochanter while its presence was observed in the femoral head.

Although this may partially explain the variation in the skeletal location of potentially persistent epiphyseal scars, it does not explain the obliteration of the epiphyseal scar in the medial clavicle, a feature which has been widely reported in the age estimation literature as this centre of ossification may be classified as a pressure epiphysis (Kreitner *et al.*, 1998; Schmeling *et al.*, 2004; Schulz *et al.*, 2005; Mühler *et al.*, 2006; Kellinghaus *et al.*, 2010). As the epiphyseal scar forms within the trabecular bone, it is suggested that the absence of a persistent epiphyseal scar in the medial clavicle may be due to the morphology of the medical clavicular epiphysis as a small cortical flake without significant trabecular bone (Scheuer and Black, 2000; Scheuer, 2002).

When assessing the potential relationship between mechanical loading and the persistence or obliteration of the epiphyseal scar, it is necessary to consider the potential sources of loading to which each skeletal area is exposed. This study found that the total persistence rate within the distal femur and proximal tibia was greater than in the distal tibia or either of the bones of the upper limb. Although

this may initially provide evidence which contradicts the proposed mechanical loading paradigm, it may be explained by the mechanostat principle (Frost, 1987; Turner, 1991; Frost, 2003). This paradigm suggested that the stimulation of bone remodelling by mechanical loading is mediated by a genetically pre-determined set point (Frost, 1987; 1998b). As the human skeleton is adapted to a bipedal stance and the bones of the lower limb are habituated to relatively high levels of mechanical loading arising from the cumulative weight of the trunk, head and neck and upper limbs in addition to the large muscle masses of the thigh and leg, it is reasonable to hypothesise that the "mechanostatic" set point of the lower limb may be greater than the upper limb (i.e. a greater deviation in the degree of applied load may be required to stimulate a change in bone remodelling rate in the bones of the lower limb than those of the upper limb) (Schoenau *et al.*, 2002). . This may partially explain the higher levels of persistence of the epiphyseal scar observed in the lower limb when compared with those of the proximal humerus and distal radius. In contrast, as the upper limb is not habituated to high levels of mechanical loading through weight bearing, the effective mechanostatic set point may be lower, thereby rendering bone remodelling within this limb more susceptible to alteration through variation in the degree of applied load.

In addition to the overall degree of mechanical loading to which bones are exposed, it is necessary to consider the trajectory of the force which passes as this may alter the rate of bone remodelling within discrete regions of the bone. The biomechanics of the skeleton have been widely studied, although a greater emphasis has been placed on those skeletal areas in which joint replacement is common for example the knee and hip (Engin and Korde, 1974; Volz *et al.*, 1980; Palmer and Werner, 1984; Högfors *et al.*, 1987; Bruns and Rosenbach, 1990; Karlsson and Peterson, 1992; Patterson and Viegas, 1995; Schuind *et al.*, 1995; Berger, 1996; Duda *et al.*, 1997; Bendjaballah *et al.*, 1998; Hurwitz *et al.*, 1998; Fyhrie and Kimura, 1999; Ulrich *et al.*, 1999; Woo *et al.*, 1999; Fukuda *et al.*, 2000; Burgers *et al.*, 2008; Koo and Andriacchi, 2008; Michael *et al.*, 2008; Cristofolini *et al.*, 2010; Kleipool and Blankevoort, 2010; Kutzner *et al.*, 2010; Koo *et al.*, 2011). Many studies relating to the biomechanical properties of bone consider the transmission of force through a single joint or bone area and generally do not

reflect the path of mechanical loading through the entirety of a bone (Engin and Korde, 1974; Bruns and Rosenbach, 1990; Anderson *et al.*, 2006; Burgers *et al.*, 2008; Koo and Andriacchi, 2008; Michael *et al.*, 2008; Matricali *et al.*, 2009; Kleipool and Blankevoort, 2010; Kutzner *et al.*, 2010; Koo *et al.*, 2011). While this may not affect the interpretation of the data pertaining to the distal radius, proximal humerus or distal femur, the explanation of the data relating to the persistence of the epiphyseal scar in the proximal and distal tibia may be more problematic.

As a single structure, forces applied to the proximal tibia must be transmitted through the bone to the ground. Similarly, in accordance with Newton's third law of motion, the distal tibia is subject to an equal load, the ground reaction force, which will be transmitted proximally through the tibia (Lenzen, 1937). No studies have been located however which consider the biomechanical properties in relation to the trajectories of mechanical loading in the complete tibia. It is therefore necessary to consolidate the extant literature with an overall theoretical model based on the findings of this study.

It has been reported that the majority of force applied to the proximal tibia follows a trajectory which passes through the medial tibial plateau (Johnson *et al.*, 1980a; Hsu *et al.*, 1990; Tsuji *et al.*, 2001; Eckstein *et al.*, 2009). This study indicates however that the greatest level of obliteration of the epiphyseal scar in this anatomical location occurs in the lateral third of the bone. Based on the morphology of the lateral tibial condyle, it is suggested that the application of force over a small contact area results in a mechanical loading of sufficient severity to stimulate the remodelling of the epiphyseal scar in this area (Koo *et al.*, 2011). The pattern of increasing BMD reported in the literature suggests that the load applied to the lateral side of the bone is transferred to the medial cortex (Khodadadyan-Klostermann *et al.*, 2004). The pattern of obliteration observed in the distal tibia supports the proposed trajectory of caudally directed mechanical loading (Figure 10.1); however this observation does not concur with the reported trajectory of mechanical loading of the distal tibia which, it has been reported, undergoes maximal loading in the antero-lateral region of the joint space (Suckel *et al.*, 2010).

Figure 10.1: Proposed mechanical load transmission through the adult tibia

Although the greatest obliteration of the epiphyseal scar in this anatomical area occurred in the medial third in both sex cohorts, the region in which the maximum persistence was observed differed between females and males. In male individuals, this was observed in the central region, while in females the lateral third of the bone exhibited the greatest mean persistence of the epiphyseal scar. The absence of a statistically significant difference in the persistence of the feature in the central and lateral regions of the distal tibia in male individuals however may suggest that the mechanical loading to which these regions are exposed may be similar. The remodelling of the epiphyseal scar in the central and lateral regions of the distal tibia corresponds to the area of the joint that is reported to be exposed to maximal loading (Suckel *et al.*, 2010). Based on the findings of this study and the proposed mechanical loading paradigm, it is suggested that the force to which the lateral region is exposed may be less than that applied to the medial third of the bone. This may be partially attributable to the effect of footwear on the transmission and dispersion of ground reaction force as some footwear types may transmit more or less force to the regions within the foot and therefore the ankle, for example training shoes are often specifically designed to minimise the forces to which the foot is exposed during physical activity (Bates *et al.*, 1983; Hardin *et al.*, 2004). In contrast, high heeled shoes may be more likely to transmit a greater degree of the ground reaction force to the foot and ankle (Barkema *et al.*, 2012).

The absence of a statistically significant relationship between the persistence of the epiphyseal scar and biological sex or side of the body, or additionally chronological age in the case of the distal radius, suggests that variation in the remodelling of the epiphyseal scar may be largely related to factors other than those directly considered by this study. It is suggested that the pattern of persistence and obliteration of the epiphyseal scar observed in the distal radius and proximal humerus may reflect the distribution of extrinsic and intrinsic forces to which the bones of the upper limb are exposed. The literature relating to the biomechanics of the upper limb, and in particular of the forearm are limited by their use of specimens devoid of soft tissue (with the exception of the interosseous membrane) (Shaaban *et al.*, 2006); however it is generally accepted that the majority of force applied to the forearm passes through the central-lateral portion of the radius in its articulation with the scaphoid (Patterson and Viegas, 1995; Schuind *et al.*, 1995). This study suggests that the remodelling of the epiphyseal scar in the lateral third of the distal radius is statistically indistinct from that observed in the medial third; however a slightly lower mean RPS value was found in this region relative to the lateral third of the bone.

In the context of the proposed mechanical loading paradigm, these findings suggest that the force applied to the medial and lateral aspects of the distal radius are statistically equivalent. As there appears to be a consensus within the literature regarding trajectory of force transmission through the wrist joint, it is proposed that the mechanical load applied to the medial third of the radius occurs by placing the interosseous membrane under tension (Markolf *et al.*, 2000; McGinley and Kozin, 2001; Standring, 2008). There is no description of inter-sex variation in the strength of the interosseous membrane, however a potential link between the strength of this membrane and chronological age has been reported (McGinley and Kozin, 2001). As no statistically significant variation was observed in the persistence of the epiphyseal scar in the medial third of the distal radius, it is proposed that biological sex does not influence the role of the interosseous membrane in the transmission of mechanical loading.

The potential role of mechanical loading on the persistence or obliteration of the epiphyseal scar is further supported by the results derived from the analysis of the

proximal humerus. In a similar pattern to that observed in the distal radius, the medial third of the proximal humerus was not found to exhibit a statistically significant degree of variation in the persistence of the epiphyseal scar between females and males. As the medial third of the proximal humerus solely constitutes the articular surface, this region is not subjected to direct mechanical loading through muscular action; however the intra-articular pressure, generated through joint contact force or ligament force of the glenohumeral joint may be sufficient to stimulate bone remodelling (Högfors *et al.*, 1987). The absence of a statistically significant difference between females and males may be explained by the functional role of intra-articular pressure in maintaining joint stability and location.

In contrast to the medial aspect, the presence of statistically significant variation in the persistence of the epiphyseal scar in the lateral third of the proximal humerus suggests that bone remodelling within these sites may be influenced by a factor or factors related to the sex of the individual. It has been noted in the literature that male individuals are likely to exhibit a higher muscle mass than female individuals, particularly in the upper limb (Frontera *et al.*, 1991; Janssen *et al.*, 2000; Abe *et al.*, 2003). As the lateral aspect of the proximal humerus facilitates the attachment of muscles of the rotator cuff, the effect of variation in the quantity and strength of muscles in these regions may vary between individuals and between sexes and age groups (Doherty, 2001; Standring, 2008). This may partially explain the variability in the persistence of the epiphyseal scar observed within this third of the bone.

Although the mechanical loading histories of the individuals included in this study could not be known, the active omission of individuals with recorded musculoskeletal disorders from the sample set goes some way to establishing a baseline level of persistence of epiphyseal scars under '*normal*' loading conditions. Through the analysis of the persistence of epiphyseal scars within discrete regions of five anatomical areas, this study has challenged the existing conceptions regarding this feature and has established positive lines of future research, which, if pursued will further augment the body of literature relating to epiphyseal scars.

11 Conclusions

This study presented data derived from the analysis of a number of current approaches to skeletal age estimation, namely the validity of extant methods of age estimation from the foot and ankle and the applicability of the obliteration or persistence of the epiphyseal scar as a criterion in radiographic methods of skeletal age assessment.

The work conducted during the initial phase of this research suggests that although the number of methods of skeletal age estimation from the juvenile foot and ankle are limited, practitioners must be circumspect in their choice of method. While the "*Radiographic Atlas of Skeletal Development*" (Hoerr *et al.*, 1962) has been shown to produce accurate and reliable estimated ages, the "*Scoring System for Estimating Age from the Foot Skeleton*" (Whitaker *et al.*, 2002) does not. Based on the findings of this study, it is recommended that in the case of the recovery of human remains limited to the foot and ankle, only the radiographic atlas (Hoerr *et al.*, 1962) method is suitable for application. In addition to the original aims of this phase of research, it was noted that when viewed radiographically, the timings of the appearance and fusion of the proximal epiphysis of the fifth metatarsal may assist the practitioner in the assessment of skeletal age from the foot. As skeletal age assessment from the juvenile foot and ankle is a relatively sparse area of literature, this study has enhanced the collective knowledge relating to this area of anthropological study; however further validation of the approaches to skeletal age assessment considered in this thesis is required to reinforce their application in forensic practice.

Following observations made during the initial research phase, and through subsequent forensic case work relating to the observation of a persistent epiphyseal scar in the distal tibia (see Appendix A), the aim of the secondary research phase was to establish the potential persistence of the epiphyseal scar in adult individuals. Through the assessment of radiographic images of five skeletal areas, this study has shown that although the epiphyseal scar may undergo a degree of obliteration over time, some remnant of the feature may remain observable in a large proportion of the population. Contrary to the previously held

belief which suggested that the observation of an epiphyseal scar was indicative of recent epiphyseal fusion and therefore a younger chronological age, this study has shown, through statistical analysis, that the relationship between chronological age and the level of persistence of the epiphyseal scar is weak. Additionally, it has been shown that neither biological sex nor side of the body exhibit strong relationships with the persistence or obliteration of the feature.

The findings of this study suggest that a paradigm shift is required in the consideration of the epiphyseal scar. This study has shown that the level of persistence of this feature is dependent on a complex interplay between multiple factors. Although in some skeletal regions (distal femur, proximal tibia and distal tibia), the combined influences of chronological age, biological sex and side of the body may exert statistically significant influences on the level of persistence or obliteration of the epiphyseal scar, the absence of these interactions in the epiphyseal scars of the upper limb suggests that the persistence of the feature is modulated by local rather than systemic influences. This was further supported by subsequent analyses of the persistence of the epiphyseal scar within discrete regions of each bone. The patterns of persistence and obliteration observed in each of the skeletal areas considered by this study suggested that obliteration of the epiphyseal scar may be greatest in regions under high levels of mechanical loading.

As suggested by the mechanostat principle and the theory of bone functional adaptation, the response of bone under mechanical loading is to align along the trajectory of maximum force. Based on the findings of this study, it is proposed that the stimulation of bone remodelling and subsequent maintenance of bone material in regions under high mechanical loading results in a differential rate of obliteration of the epiphyseal scar within a given anatomical region. The proposed model may also explain the observed variation in the persistence of the epiphyseal scar between skeletal areas and in particular, between those areas represented by the upper and lower limbs; and the absence of epiphyseal scars from apophyseal regions such as the calcaneus, olecranon and iliac crest. In addition, the hypothetical paradigm may explain the weak relationships observed between chronological age, biological sex and side of the body through their interaction

with skeletal muscle mass and strength and the associated relationship between these factors and mechanical loading of the skeletal system.

Through the examination of the persistence of the epiphyseal scars of multiple anatomical sites in adult individuals, this study has augmented the available literature relating to the potential persistence of the epiphyseal scar. Based on the findings of this study, an alternative hypothesis regarding the observed pattern of persistence or obliteration of the feature in adult individuals is proposed, namely that the level of persistence or obliteration of the epiphyseal scar is dependent on the complex interaction of numerous factors including those related to systemic and localised variables (e.g. hormonal variation, mechanical loading) and is not directly associated with the chronological age of the individual

Through the assessment of the approaches to skeletal age estimation from the juvenile foot and ankle published by Whitaker *et al*. (2002) and Hoerr *et al.* (1962), this study has shown that only the method of Hoerr *et al*. (1962) is of sufficient reliability and accuracy to be utilised in the assessment of skeletal age in the forensic context. Therefore, the first hypothesis of this thesis has been partially upheld in respect of the "Radiographic Atlas of Skeletal Development of the Foot and Ankle" ; and partially rejected, in respect of the "Scoring system for estimating age in the foot skeleton" (Whitaker *et al.*, 2002).

With reference to the second hypothesis of this thesis, it has been shown that the epiphyseal scars of the proximal humerus, distal radius, distal femur, proximal tibia and distal tibia, although they may undergo a degree of obliteration, are likely to persist to some degree in the majority of individuals. In addition, the level of obliteration or persistence of the epiphyseal scars in each of these regions has been found to be largely independent of the chronological age of the individual. The findings of this study therefore reject the stated hypothesis relating to the obliteration of epiphyseal scars in adult individuals.

The results of this study have potentially far reaching consequences for the application of the obliteration of the epiphyseal scar as a maturity criterion in radiographic approaches to skeletal age estimation in certain anatomical areas. This may be of particular importance in methods of skeletal age assessment which

are commonly utilised, such as those pertaining to the hand and wrist, knee and foot and ankle.

12 Future Work

This section will consider the work that may be undertaken for which the findings of this study acts as the foundation.

12.1 Skeletal age estimation from the juvenile foot and ankle

It was apparent from the research undertaken in this field that there is a distinct paucity of published sources relating to skeletal age estimation from the juvenile foot and ankle.

Unlike other anatomical regions, where approaches to skeletal age estimation have evolved to include new techniques of medical imaging such as CT (Kreitner *et al.*, 1998; Schulz *et al.*, 2005; Kellinghaus *et al.*, 2010) and MRI (Dvorak *et al.*, 2007b; Schmidt *et al.*, 2007b; Hillewig *et al.*, 2011), skeletal age estimation from the foot is currently restricted to plain film radiography (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002). This study has found the "*Radiographic Atlas of Skeletal Development of the Foot and Ankle"* (Hoerr *et al.*, 1962) is the only published method for estimating age from the juvenile foot and ankle of sufficient reliability and accuracy to be considered for application in a forensic context. As this method is based on plain film radiography, the standards presented within the atlas (Hoerr *et al.*, 1962) may not be directly applicable to CT slice images. It is therefore proposed that the "*Radiographic Atlas of Skeletal Development of the Foot and Ankle*" (Hoerr *et al.*, 1962) be tested against CT slice images to establish the validity of the cross-application of methodologies between imaging modalities. If the radiographic atlas were found to be inappropriate for use on CT, this may lead to the establishment of an atlas specifically for application with CT images.

Although this study has augmented the extant literature pertaining to skeletal age estimation from the juvenile foot and ankle, further work is required to establish a significant body of information on which an estimation of age from this skeletal region may be based. As a result of the work conducted during the first phase of this thesis, it is proposed that greater attention be paid to the timing of appearance and fusion of the proximal epiphysis of the fifth metatarsal and that further work

be undertaken in relation to its inclusion within approaches to skeletal age estimation.

12.2 The persistence of epiphyseal scars in adult individuals

As the first study to examine the persistence of epiphyseal scars in multiple anatomical regions within a modern radiographic sample, the findings of this thesis may be considered the baseline of the persistence of epiphyseal scars in these anatomical regions in this population. Consequently, this study set out to establish the validity of the presumed relationship between chronological age and the level of obliteration or persistence of the epiphyseal scar when viewed through plain film radiography. Although the findings of this study indicate that these factors are not intimately related, it is proposed that obliteration of the epiphyseal scar occurs as a result of mechanical loading. It is therefore suggested that this work be continued and the relationship between mechanical loading and the persistence or obliteration of epiphyseal scars be considered in greater detail. This may include a comparison of the persistence of epiphyseal scars between pressure epiphyses and traction epiphyses. Evaluation of the effect of mechanical loading on the persistence of the epiphyseal scar may also provide some information relating to any potential function of the epiphyseal scar. To enable a comparison with this initial study, it is suggested that the first phase of any future work in this field also be conducted using radiographs. Subsequent studies may also include the examination of epiphyseal scars through the evaluation of CT slice images.

As it is proposed that remodelling of the epiphyseal scar may result in its obliteration, further research considering the trabecular structure surrounding the epiphyseal scar could be undertaken through the application of micro-computed tomography (μCT) on dry bone samples. This would facilitate a quantitative examination of the trabecular structure, number and geometry within the region of the epiphyseal scar and allow a comparison to be made between the region encompassing the epiphyseal scar and those proximal and distal to it. As the proposed mechanical loading paradigm is reliant on the response of bone to adapt an optimal morphology, it would be expected that a significant difference in the degree of anisotropy would occur within the trabecular bone in the vicinity of the

epiphyseal scar in a pattern that would mirror that observed in the level of persistence of the feature when viewed radiographically.

This study forms the cross-road for numerous possible research avenues with the potential to enhance the literature in their respective fields. Through the application of repeatable assessment methods, this study has reinforced the importance of falsifiability in scientific research and the notion that continuous testing is required to ensure that only those methods found to be based on sound foundations are applied in forensic practice.

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Appendix A Case Study

A.1 Case report

Between March and June 2011, human remains from an adult male were recovered from a series of locations in eastern England over a period of several months. The remains were dismembered at the proximal femora, proximal humeri and cervical portion of the vertebral column. Although the complete remains of the individual were eventually recovered, at the time of initial consultation with forensic anthropologists only the left lower limb had been recovered. Consequently, the estimation of chronological age was based solely on this anatomical region. The left arm and torso of the individual were recovered within a week of the initial find however the right arm, right leg and head of the individual were not located until June 2011. Identity had been established by DNA analysis prior to the recovery of the remainder of the body and so further anthropological analysis was not necessary.

The lower limb was examined by a forensic pathologist and although the sex and body size of the individual could be determined from the remains, advice was sought regarding the chronological age of the decedent. As part of the post-mortem examination, CT was used to image the remains. A single CT slice, from which an estimation of chronological age was made based on available standards and methods, was forwarded to the forensic anthropologists. The following report details the results of the age assessment carried out on the remains of the left lower limb prior to the confirmation of identity and the observations made during the examination of the radiographic images. The implications of these observations will be discussed in the wider context of age estimation.

A.2 Methods

The estimation of chronological age relies on the assessment and interpretation of biological changes within the skeleton. This can be carried out through the physical examination of the gross morphology of remains or through assessment of radiographic images. In juveniles and young adults, it is possible to determine the chronological age of an individual based on the degree of ossification and fusion of

the primary and secondary ossification centres. Once fusion of the epiphyses to their respective diaphyses is complete, the chronological age of an individual becomes more difficult to ascertain with precision as degeneration of the skeleton is less predictable due to the numerous extrinsic factors which exert an influence on the adult skeleton.

A.1.1 Age estimation from the foot and ankle

Methods used to estimate age from the foot and ankle are few in number and are restricted to assessing the chronological age of sub-adult individuals (Hoerr *et al.*, 1962; Whitaker *et al.*, 2002). Through charting the development of the ossification centres for the foot and that of the distal epiphyses of the tibia and fibula, the chronological age may be estimated until epiphyseal fusion is complete and the bones have adopted their adult morphology. Fusion of the distal tibia, as viewed on the dry bone, is reported to occur between the ages of 14 and 18 years in females and 16 and 20years in males (McKern and Stewart, 1957; Hoerr *et al.*, 1962; Scheuer and Black, 2004).

The final maturity indicator observable in this region is the epiphyseal scar, a persistent transverse radio-opaque line in the region of the former tibial growth plate (Hoerr *et al.*, 1962). This is generally considered to become obliterated through the normal process of bone remodelling, and consequently its presence is associated with individuals in whom epiphyseal fusion has recently completed.

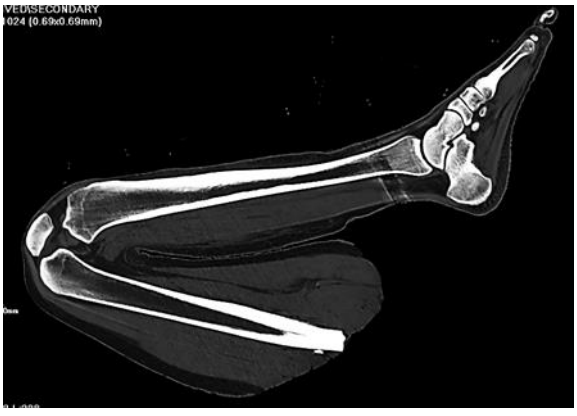

Figure A. 1: CT slice of the left lower limb

Examination of the CT slice obtained from the left leg of the individual (Figure A.1) whose remains were recovered were examined and age was estimated according

to the radiographic standards published by Hoerr *et al*. (1962). The limb did not appear to display any distinguishing features; however the presence of bright epiphyseal scars was noted at the distal femur, proximal tibia (Figure A. 2) and distal tibia (Figure A. 3).

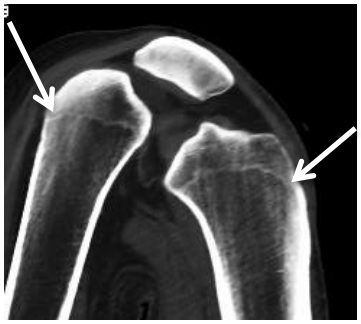

Figure A. 2: Epiphyseal scars present in the distal femur (left) and proximal tibia (right)

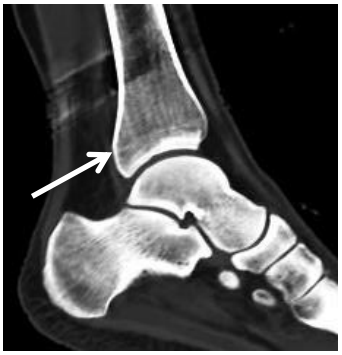

Figure A. 3: Epiphyseal scar present in the distal tibia

The image of the distal tibia resembled the last radiographic plate within the Radiographic Atlas of Skeletal Development of the Foot and Ankle (Hoerr *et al.*, 1962) which corresponds to 18 years of age. Consequently, based on this region alone, an estimated age of late teens to early twenties was considered and confirmation was given that this could be from a young adult.

A.1.2 Age Estimation from the knee

Estimation of age from the knee comprises the assessment of both the proximal tibia and the distal femur. As with the distal tibia, assessment was carried out using the atlas "A Radiographic Standard of Reference for the Growing Knee" (Pyle and Hoerr, 1969) although other methods are available (O'Connor *et al.*, 2008; Cameriere *et al.*, 2012). The presence of the epiphyseal scar is noted at both the proximal tibial and distal femoral growth plates in all three of these methods;

however its temporal stability is a matter of contention. It is important to note that although age assessment from radiographic images is a technique which has been applied throughout the last century, the use of CT imaging in the forensic context is in its relative infancy. As a result, there are currently no methods of age assessment from the knee developed specifically for use in this imaging modality. Any assessments of age made using a method on an imaging modality other than that from which it was developed are therefore limited in their application.

During the assessment of the knee, fusion at both growth plates was complete and bright epiphyseal scars were observed. The pathologist had also stated that the growth plate was visible externally. This led to the conclusion that fusion had recently occurred and therefore a minimum age of "late teens" was appropriate, although no maximum age was possible. To give an appropriate estimation of age based on the CT slice data obtained from the lower limb, the assessed ages of the knee and the ankle were combined to provide a representative age range based on the skeletal morphology. From these assessments, an estimated age range of late teens to early twenties was confirmed as possible to the forensic pathologist and investigating police force.

Following confirmation of identity via DNA testing, the chronological age of the decedent was released as 33 years of age. The estimated age of the individual was therefore significantly younger than the known chronological age. The interpretation of the osteological evidence in this case was based on currently accepted standards of age estimation.

A.3 Discussion

The assessment of age in this case was carried out through the use of radiographic standards of reference for the ankle and knee (Hoerr *et al.*, 1962; Pyle and Hoerr, 1969). The error in estimation of approximately ten years may be attributed to the observation of the epiphyseal scars at the growth plates of the lower limb. Based on currently available standards, the presence of epiphyseal scars was considered an indication of relatively recent fusion of the epiphyses to their diaphyses and therefore suggestive of a younger chronological age.

The association between epiphyseal scars and younger individuals is based on the assumption that osseous remodelling causes alteration to the trabecular structure in the region of the former growth plate, resulting in the apparent equalisation of radiographic density. Until this point however, no quantitative evidence has been published to support this hypothesis. The persistence or obliteration of epiphyseal scars has been a matter of contention throughout the twentieth and into the twenty-first centuries with proponents of persistence such as Cope (1920) being outweighed by those who favoured the age related obliteration thesis of T. Wingate Todd (1930) and his successors (Greulich and Pyle, 1959; Hoerr *et al.*, 1962; Pyle and Hoerr, 1969) .

The case presented in this report has highlighted a void within the currently available age estimation literature regarding the potential persistence of epiphyseal scars and the subsequent impact on the forensic estimation of age. Without modifications to the currently available literature to include studies that quantify the persistence of epiphyseal scars, errors in estimated age such as that which occurred in this case will continue. It should also be noted that the application of radiographic standards of age estimation to imaging modalities for which they were not designed may not be appropriate. Further research is required to establish the validity of cross application of studies to modern types of medical imaging.

This study marks a departure from the conventional interpretation of the epiphyseal scar as an indicator of recent epiphyseal fusion and underlines the necessity for methods used in forensic investigation to be reviewed and if necessary, altered to ensure that misidentification does not occur. The observation of epiphyseal scars in the long bones of an adult individual in combination with recently produced literature suggests that this is a region of anthropological study which would benefit from further research. Although inferences may be drawn from this case regarding the persistence of epiphyseal scars in adult individuals, there are certain limitations which must be considered during the extrapolation of the findings of this case to the wider remit of anthropological age assessment.

A.4 Limitations

The majority of methods of age estimation routinely used in forensic cases in the UK were developed as a means of monitoring the normal development of children though radiographic assessment. Consequently, they are based on plain film radiographs. Unlike the more modern imaging techniques of computed tomography, plain film x-rays are a 2 dimensional representation of a 3 dimensional structure. Superimposition and angular distortion must therefore be accounted for in the interpretation of the image. This therefore limits the degree to which characteristics such as bone density can be interpreted.

The use of CT imaging allows a three dimensional representation to be reformed from slice data, enabling an appreciation of the bone density to be made as no overlap occurs. There is no data which compares the efficacy and biases which are introduced by the differing imaging modalities and it is considered that the differences between plain film and CT therefore require separate standards of age estimation to be developed for each imaging modality. At present, the application of CT to skeletal age estimation is limited by the legal and ethical boundaries related to non-therapeutic x-radiation exposure; however some methods have been devised for its use in age estimation from the medial clavicular epiphysis (Schulz *et al.*, 2005; Kellinghaus *et al.*, 2010).

As radio-opaque lines represent areas of increased density relative to the surrounding bone, it could be suggested that the radio-opaque lines would be observable in other imaging modalities including plain film radiographs. As the resolution of CT imaging is greater than that of conventional radiography however, the perceived density of the line may appear greater in CT than in a single plain film radiograph (Mühler *et al.*, 2006). This could lead to the misinterpretation of any line observed within the image and as a consequence an inaccurate estimation of age.

Appendix B Ethical approval for the use of clinical images in study phase 1

>>> "Hunter Stewart (NHS TAYSIDE)" <stewart.hunter@nhs.net> 13/01/2011 12:57 >>>
Steve/Luciana
I confirm that as the images used were fully anonymised the use of the x rays for research purposes was permissable.
Stewart Hunter
Information Governance Coordinator
01382 660111 x 33472
0782 568 0599

From: Menhinick Stephen (NHS TAYSIDE)
Sent: 11 January 2011 09:06
To: Lucina Hackman; Hunter Stewart (NHS TAYSIDE)
Subject: RE: hello

Hi Stewart,

A couple of years ago Lucina, who works at the department of forensic anthropology with Professor Sue Black, started a research project looking at x-rays of wrists and ankles of people under the age of 20 to look at the correlation between bone development and age. At that time I asked you if this would be acceptable and you agreed providing that all patient information was removed and any images to be used would be fully anonymised. As you will see below Lucina has now completed her research and would like to have a note to say that the information has been obtained legitimately. Would you be able to provide this?

Many thanks,

Steve

Stephen Menhinick
Senior Radiographer
Department of Clinical Radiology
Ninewells Hospital
Dundee
DD1 9SY

Appendix C Ethical approval for the use of clinical images in study phase 2

CONFIDENTIALITY STATEMENT - for users of person identifiable data

NHS Tayside

User Details

Name:	Catriona Davies
Position:	PhD. Student
Organisation:	CAHID, University of Dundee
Address:	Centre for Anatomy and Human Identification, MSI/WTB Complex, University of Dundee Dow Street DD1 5EH
Tel:	01382384210

Sponsor Details

Name:	Dr Gavin Main
Position:	Consultant Radiologist
Organisation:	Ninewells Hospital, NHS Tayside
Address:	Radiology Department, Ninewells Hospital Dundee DD1 9SY
Tel:	01382632966

Data Protection Reg. No. Z6650266

Data Requested : A Data Processing Specification must also be completed.	Radiographic images of ankle, knee, hip, wrist, and shoulders of males and females between 20 and 70 years of age. 50 radiographs of each sex at within each 10 year cohort are required (2500 total). Only date of birth and date of image, and the sex of each individual will be recorded and will be stored separately from images which will be assigned a coded unique reference number. Radiographs will be visually scored for the presence of epiphyseal scars, and the appearance of the scars will also be scored and coded.
Co-Users of the Data :	Prof. Sue Black, Director, Centre for Anatomy and Human Identification (Supervisor), University of Dundee; Miss Lucina Hackman, DVI Co-ordinator and advisor, Centre for Anatomy and Human Identification, University of Dundee
Intended use of data (inc. publications) :	PhD. Study investigating the persistence of epiphyseal scars in relation to chronological age with the purpose of furthering forensic age estimation practices. Publications are likely but no data will be used directly, only the results of the study will be presented in any publications

User's Declaration

I declare that I understand and undertake to abide by the rules for confidentiality, security and release of data received from NHS Tayside.

Signature Catriona Davies

Date 11/11/2011

Sponsor's Declaration (to be signed by a consultant if patient data is requested and the applicant is not of that status or is not medically qualified)

I declare that the above named user of the data is a bona fide worker engaged in a reputable project and that the data requested can be entrusted to this person in the knowledge that they will conscientiously discharge their obligations in regard to confidentiality of the data.

Signature G.MAIN

Date 6/9/11 CONS. RADIOLOGIST

On completion, please return this form to:

For NHS Tayside use only

Information Governance Officer
NHS Tayside
Ashludie Hospital
Monifieth
Dundee
DD5 4HQ

Release authorised by
Date
Ref.No.

NHS Tayside

RULES ON CONFIDENTIALITY, SECURITY AND RELEASE OF INFORMATION

FOR USERS OF NHS PATIENT DATA

1) If the data received from NHS Tayside are to be held on computer, the signatory of this request, or the organisation (s)he represents, should have an appropriate registration with the Office of the Data Protection Registrar. Details of the registration number should be entered on this document.

2) Data received from NHS Tayside must not be used for any purpose other than for the intended use specified on this document.

3) Data received from NHS Tayside must not be divulged to any person who is not specified as a 'co-user of the data' on this document.

4) Proper safeguards should be applied in keeping the data and destroying it on completion of the work/project declared to prevent any breach of confidentiality.

5) Any misuse or loss of these data should be notified immediately to the Information Governance Officer for NHS Tayside at Ashludie Hospital, Monifieth (01382-527920).

6) Recipients of information supplied by NHS Tayside are reminded that the data has been supplied for the purposes stated in the approved study only. Further submission for approval will be required for any other uses of that data.

7) Any statistics or results of research based on data received from NHS Tayside should not be made available in a form which:
   a) directly identifies individual data subjects
   b) is not covered by the 'intended use of data' specified

NHS Tayside would welcome copies of any publications based on data supplied.

Information Governance
Ashludie Hospital
Monifieth
DD5 4HQ

Telephone : 01382 527920
Fax : 01382 527808

NHS Tayside

CALDICOTT APPROVAL - DATA PROCESSING SPECIFICATION

To be submitted with application for Caldicott Approval

For **each separate source** of patient identifiable data that you intend to access in support of your study please provide the following information.

Data Source: (*Medical Records/System Name)*
CRIS Radiographic Database

Data Items: (list the data items that you will require from the named data source)			
Ankle Films (Male and Female 20-70 years)	Knee Films (Male and Female 20-70 years)	Hip Films (Male and Female 20-70 years)	Wrist Films (Male and Female 20-70 years)
Shoulder Films (Male and Female 20-70 years)			

Data Source Contact Details: (who have you agreed access to the source data with?)	
Name: Dr Gavin Main	Designation: Consultant
Base: Radiology Department, Ninewells Hospital	Tel No: 01382632966
Email address: gavin.main@nhs.net	

Data Storage Arrangements: (where arrangements are described in a supplied study protocol then reference to the relevant sections of the protocol can be used)	
Location: (NHS Tayside, University, etc.) Data will be collected at the Radiology department of Ninewells hospital and analysed and stored at the Centre for Anatomy and Human Identification, University of Dundee	Device to be held on (desktop, laptop, network storage, etc.) Password protected and secure external hard drive
Access Controls (how will the data be protected from unauthorised access?) Password protection as well as physical access to the external hard drive will be limited to Catriona Davies as principal researcher, Prof. Sue Black, as supervising academic, and Lucina Hackman, advisor. The external hard drive will be stored in a locked cabinet during times when it is not being used.	Encryption: (will encryption be used to protect the data?) Password encryption will be used to protect the data from unauthorised access on the external hard drive.
Anonymisation: (how will the identity of individuals be protected) All patient information will be removed from the images prior to download from the CRIS system. The images will not be identifiable in any way. The sex, date of birth and date of image will be recorded in a record book for the purpose of calculating chronological age, and sex is required for the analysis of the data. This information will be stored separately from the	Format (spreadsheet, database, etc.) Images will be stored in files as JPEG images with unique reference codes, e.g. MRDT1 would be male right distal tibia 1. Date of birth and date of image will be stored in an excel spreadsheet and will only be related to the unique reference number. No patient will be identifiable from this number.

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images and will not be accessed by any unauthorised personnel.	

If you intend to make contact with patients identified through the processing of this data, indicate how this will be done and how you will ensure that it is appropriate to contact them. It is recommended that contact with patients is through correspondence signed by the patient's GP/Clinician or Head of Clinical Service.

Appendix D Publications offered in support of thesis

The following publications have been prepared from the work conducted during this studentship and presented in this volume:

Davies, C., Hackman, L. and Black, S. 2013. The utility of the proximal epiphysis of the fifth metatarsal in age estimation. *Journal of Forensic Sciences,* 58, 2, 436-442.

Davies, C., Hackman, L. and Black, S. 2013. A test of the Whitaker scoring system for estimating age from bones of the foot. *International Journal of Legal Medicine,* 127 2, 481-489.

Davies, C., Hackman, L. and Black, S. 2013. The persistence of epiphyseal scars in the adult tibia. *International Journal of Legal Medicine,* DOI: 10.1007/s00414-013-0838-3 [Epub. prior to print]