Estimation of age at death from the microscopic structure of the femur

by

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DECLARATION

I, Natalie Keough, declare that this thesis is my own work. It is being submitted for the degree of Master of Science in Anatomy at the University of Pretoria. It has not been submitted before for any other degree or examination at this or any other University.

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ABSTRACT

Estimating age at death from skeletal remains can be done with relative accuracy when a skeleton is complete, however incomplete and/or poorly preserved skeletons pose a problem in assigning an accurate age range to unknown remains. Techniques for determining age at death from the microstructure of bone have shown to be relatively accurate in North American and European populations but, until recently, had not been attempted on a South African group. This technique is based on the fact that bone remodels and changes throughout an individual's life.

The purpose of this study was to develop standards for estimating age at death, using bone microstructure, that are applicable to a South African population. The sample consisted of 146 individuals (105 males and 41 females) of known age and sex. A 0.2 cm x 1.0 cm sample was removed from the anterior surface of the femur, and slides were prepared according to standard histological methodology. Ten variables, which included the total osteon count (measurable and non-measurable), the average Haversian canal diameter, the average number of lamellae per osteon, the total number of osteon fragments, the number of non-haversian canals, the total number of resorption spaces and the average percentage of osteonal bone, unremodeled bone and fragmental bone were assessed. The relationship between the changes in each of the variables with age was examined. Four variables demonstrated significant correlation with age and included the total osteon count (r = 0.53), the percentage unremodeled bone (r = -0.53), the total number of non-haversian canals (r = -0.55) and the average percentage of fragmental bone (r = 0.55). These variables were then used to calculate single and multiple linear regression formulae to determine age. Coefficient of determination (r^2) for multiple regression analyses ranged from r^2 = 0.27 to 0.42. The general range of the standard error of the estimate (SEE) for this study ranged between 13.31 and 14.04 years and is similar to the results of previous studies. Various factors may have contributed to low r^2 values, such as poor nutrition, mechanical stress to the bones and misreporting of age of the individuals in the sample. The microscopic techniques appear to not be more accurate than macroscopic methods for estimating age at death, but can be useful in situations were remains are fragmentary or in combination with macroscopic methods.

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A special thanks goes out to my parents and my family who have supported me throughout my studies, through good times and bad times. Thank you for giving me the ability to never give up and to achieve anything I set my mind to.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter: 1 Introduction

1.1. General

Physical anthropologists are constantly faced with the task of identifying unknown skeletal remains, whether it be in an archaeological or forensic context. The requirements for identification of an unknown individual include certain demographic characteristics such as age and sex, which can be determined by the use of established morphological and metrical techniques. Factors of individualization (eg., stature, pathology, teeth mutilations) are useful in narrowing down the field of possibilities. The ability to determine these characteristics comes from the variation observed in the human skeleton and, specifically in the case of age at death estimation, the methods used are based on evaluating sequential changes in growth and degeneration in the skeleton from juvenile to adult. Estimating age at death from skeletal remains can be done with relative accuracy when the skeleton is complete, however incomplete and/or poorly preserved skeletons as well as adult individuals pose a problem in assigning an accurate age range to unknown remains. Estimates of age should be based on more than one indicator and due to the occurrence of incomplete skeletal remains there should be a variety of ageing methods available for various elements of the skeleton (Stout *et al.,* 1994).

The crime rate in South Africa between 1997 and 2000 was increasing at an alarming rate thus prioritizing the popularity of research in the field of forensic anthropology (Steyn *et al*., 1997; Steyn and İşçan, 1999). The crime statistics in 2001 seemed to show a steady decrease in crime leaving it stable but at a very high rate (Schöteich, 2002). In 1995 alone it was found that Gauteng had 2,008 reported, unknown persons varying from non-decomposed to skeletonized (Steyn *et al*., 1997). The need for development of osteological standards for identification of these remains has led to a much needed increase in the amount of research in this area (Steyn and İşçan, 1999; Patriquin *et al*., 2002).

Problems fuelling this dilemma of unidentified bodies include the rapid increase in the number of illegal immigrants as well as low socio-economic status, which results in many of the people in this country, legal or illegal, not having dental records (Steyn *et al*., 1997). Developed countries use dental records to make personal identification, but in South Africa there is a need for other methods to be developed. Since the majority of the standards used for identification of skeletal remains are

based on groups that are international, there is a dire need for South African standards to be developed.

1.2. Age at death estimation

Various methods for estimating age from skeletal remains have been developed over the years (Lynnerup *et al.*, 1998). Methods for determining age at death of juvenile individuals include the use of the skull (eg., Redfield, 1970; Loth and İsçan, 2000), dating of Harris lines (Maat, 1984), ossification centres (eg., Francis *et al*., 1939; Noback, 1954; Scheuer and Black, 2000; Scheuer, 2002), dental eruption (eg., Smith, 1991; Loth and İsçan, 2000; Scheuer and Black, 2000), epiphyseal closure (eg., Stevenson, 1924; Krogman and İsçan, 1986), length of the long bones (eg., Hoffman, 1979; Hunt and Hatch, 1981; Scheuer and Black, 2000) and the vertebral column (eg., Albert and Maples, 1995; Scheuer and Black, 2000). Age determination of juveniles can be made with relative accuracy. The reason for this is that the sequence of growth in juvenile individuals follows a fairly constant rate (Loth and İsçan, 2000; Scheuer, 2002).

The determination of age in adult individuals is more complex than juveniles. Skeletal changes in adults are less obvious and age-related changes of skeletal remains is not a constant phenomenon (Steyn *et al.*, 2004). The methods used for determining age at death in adult individuals include the changing morphological characteristics of the pubic symphysis (eg., Gilbert and McKern, 1973; Krogman and İsçan, 1986), the morphological characteristics of the sternal ends of the 3rd, 4th and 5th ribs (eg., İsçan *et al*., 1984, 1985; Krogman and İsçan 1986; Stout *et al.* 1994; Oettlé and Steyn, 2000), the degree of closure of the cranial sutures (eg., Meindl and Lovejoy, 1985; Todd and Lyon, 1924, 1925a, 1925b, 1925c; Krogman and İsçan 1986) , the auricular surface of the ilium (eg., Lovejoy *et al.*, 1985a; Buckberry and Chamberlain, 2002; Yuriko *et al.*, 2005) and the characteristics of the teeth (eg., Gustafson, 1950; Brothwell, 1989). The determination of age at death has primarily been dominated by the use of gross macroscopic techniques and rightly so, as these methods have proven to be relatively accurate (Maat *et al.*, 2006). The main disadvantage of macroscopic techniques is that the methods require complete skeletal elements. In cases were human remains are fragmentary, the reliability of macroscopic techniques is reduced.

In instances where the remains are fragmentary or incomplete, the application of histological methods to estimate age is seen to be more advantageous than macroscopic ones (Stout *et al.*, 1994). Microscopic techniques measure the progression of bone remodeling, a biological phenomena, that occurs at a relatively constant rate within the cortical bone throughout ones life (Maat *et al.*, 2003). At least 70% of the variation in the number of osteons, osteon fragments, number of non-haversian canals, and the percentage of unremodeled bone has been shown to have age related changes (Kerley, 1965; Ericksen, 1991; Maat *et al.*, 2006). This makes the study of the histological structure of bone and its relation to age a fascinating and worthwhile topic to pursue.

Bone histology for determining age at death has been in use for approximately 40 years. Since Kerley's dissertation in 1965 which describes the original method of estimating age at death from the microstructure of bone, several modifications and variations to this technique have been introduced. The different methods vary from one another in regard to sample location, type of bone used, amount of bone required as well as microscopic structures observed (Stout, 1998). Limitations for this technique, like any other, exist and include factors such as population variation, different age profiles among those populations, nutrition and disease (Aiello and Molleson, 1993). Techniques for determining age at death from the microstructure of bone have shown to be relatively accurate in North American and European populations but, until recently, have not been attempted on a South African group. This study is the first to examine this technique on an African, particularly South African, sample. Few studies have researched and compared the possible differences between histological structures of various populations.

The aim of this study was to develop regression formulae for estimating age at death using bone microstructure that is applicable to this population group. Different variables from previous studies were used and their relationship with age was determined.

Chapter 2: Literature review

2.1. Bone histology

2.1.1. Classification and structure of bone

Bone is a rigid form of specialized connective tissue that serves as a support, protective and locomotive system for the body. Bone is comprised of a cortex with the majority of the tissue being bone matrix (Freemont, 1998). The factor that distinguishes bone from the rest of the tissues in the body is the process of mineralization that the matrix undergoes (Ross *et al.,* 1995). The cortex surrounds a marrow cavity and trabeculae, which are defined as interconnecting bony spicules (Freemont, 1998).

Bones of the human body furthermore classifies into five categories based on its structure and shape, such as long bones, short bones, irregular bones, flat bones and pneumatic bones (Freemont, 1998). Long bones (eg., femur, tibia, and humerus) are longer in one dimension than in the other and consist of a shaft (diaphysis) and distal and proximal ends known as the epiphyses. Short bones (eg., carpals, tarsals) are almost equal in length and breadth, and flat bones (eg., skullcap) are thin and plate-like. Irregular bones (eg., vertebrae) do not display any of the characteristics seen in the above categories and the shape may be quite complex in nature. Pneumatic bones contain air sinuses (Freemont, 1998), and are found in the nasal section of the cranium.

Bone can be either cancellous (spongy) or compact (dense). Cancellous bone originates in the inner aspect of the bone and its characteristics include open spaces situated in a mesh-like network of trabeculae. In a living individual, the meshwork is continuous and occupied by marrow and blood vessels. Compact bone consists of highly dense layers of bone, located on the periphery. Metabolites are unable to diffuse through the calcified matrix of the bone and therefore the bone relies on canaliculi, which are thin, cylindrical spaces perforating the matrix, as a vehicle for communication.

Tissues containing osteogenic cells line all internal (endosteum) and external (periosteum) surfaces of bone. The periosteum serves as a transition region between the cortical bone and the overlying soft tissue (Allen *et al.,* 2004).

2.1.2. Cells present in bone tissue and their functions

2.1.2.1. Osteoprogenitor cells

Osteoprogenitor cells are also termed 'resting' cells and are derived from mesenchymal tissue. They possess the ability to transform into either osteoblasts or osteoclasts (Ross *et al.,* 1995; Leeson, 1985). Osteoprogenitor cells have a spindle like shape and line the innermost layer of the periosteum, the marrow cavities, the Haversian canals and the Volkmann's canals (Ross *et al.,* 1995).

Two types of osteoprogenitor cells are recognizable, one being the pre-osteoblast and the other being the pre-osteoclast (Leeson, 1985). Another function, apparently carried out by these cells, is that they maintain and supply the required nutrients to the embedded osteocytes (Ross *et al.,* 1995).

2.1.2.2. Osteoblasts

Osteoblasts are responsible for the laying down of the bone matrix (ground substance and collagen) and the subsequent calcification of that matrix (Ross *et al.* 1995). They are also responsible for collagen and non-collagenous protein synthesis, and the orderly organization of these proteins (Freemont, 1998). These cuboidal cells are specifically located near the surface of developing bone tissue in a formation similar to that of simple epithelium (Junqueira 1998). Osteoblasts contain only one nucleus, which is large and contains one prominent nucleolus (Leeson, 1985). Osteoblasts only deposit osteoid (initial unmineralized bone) on pre-existing mineralized surfaces (Freemont, 1998). Bone mineralization only occurs if the calcium and phosphate supplies are adequate in the extracellular fluid (Freemont, 1998). Calcium and phosphate supplies, in turn, depend upon the presence of parathyroid hormone and 1, 25 dihydroxy vitamin D_3 (Freemont, 1998). It is thus obvious that hormones will play an indirect yet vital role in the formation of bone.

Osteoblasts communicate with each other via cytoplasmic processes that extend through the osteoid and connect to the adjacent cell processes by means of gap junctions (Ross *et al.,*1995; Leeson *et al.,* 1985). The cytoplasmic processes are embedded in the matrix once the area surrounding the osteoblast has mineralized. The osteoblast is now referred to as an osteocyte (Hall, 2005) or a mature osteoblast and the tunnels formed by the embedded cytoplasmic processes are known as canaliculi.

2.1.2.3. Osteoclasts

Osteoclasts are large, multi-nucleated cells found in the region of the bone that is undergoing the process of resorption. Osteoclasts serve to resorb mineralized bone, dentine and calcified cartilage (Väänänen and Zhao, 2002). These cells are located in grooves known as Howship's lacunae, which suggests, that these lacunae are formed by the erosive activity of the overlying osteoclasts (Ross *et al.,* 1995). When the cells are examined, using an electron microscope, numerous cytoplasmic processes and microvilli on the border of the cell, facing the bone matrix, can be seen and are known as a 'ruffled border' (Leeson, 1985). A cytoplasmic zone can be observed enclosing this ruffled border and is responsible for the adhesion of the osteoclast to the bone matrix and subsequent creation of a microenvironment, in which the resorption process can occur (Junqueira, 1998).

Osteoclast activity is under the control of cytokines and hormones (Junqueira, 1998), therefore any changes occurring in these substances can affect the activity of the cells, for example, if the parathyroid hormone levels increase, there would be an increase in bone resorption and thus an increase in osteoclastic activity (Ross *et al.,* 1995).

2.1.3. Bone Formation

Bone formation can be classified into two very distinct groups and is defined as being either intramembranous ossification (direct mineralization of matrix) or endochondral ossification (bone deposition on pre-existing cartilage) (Leeson, 1985; Junqueira, 1998).

2.1.3.1. Intramembranous Ossification

This type of ossification takes place within the condensations of mesenchymal tissue. It is the source of flat bones and is responsible for the growth of short bones and thickening of long bones (Junqueira, 1998).

The first evidence of this process taking place is in the eighth week of gestation (Ross *et al*., 1995). The mesenchyme consists of primary connective tissue cells (Leeson, 1985) that differentiate into osteoblasts (Junqueira 1998; Leeson *et al.,* 1985). The osteoblasts begin laying down the bone matrix (proteoglycans and collagen) and gradually separate from each other during this process (Ross *et al.,*

1995). Once the bone matrix has surrounded the osteoblasts, it undergoes a process of calcification. These osteoblasts are now known as osteocytes (mature osteoblasts) and are located in the forming lacunae. Subsequently the primitive cells in the surrounding membrane give rise to osteoprogenitor cells (Ross *et al.,* 1995). Some of these cells, in turn, develop into osteoblasts (Ross *et al.,* 1995). During initial bone growth, these osteoblasts emerge on the surface of the developed bone, and it is through this process of apposition that the bone is able to thicken (Leeson, 1985).

2.1.3.2. Endochondral Ossification

Endochondral ossification begins in the second trimester and continues into early adulthood (Ross *et al.,* 1995). This form of ossification is responsible for the formation of short and long bones and takes place in hyaline cartilage, which already resembles a small version of the fully-grown bone (Junqueira, 1998). This process starts with a structure known as a bone collar that is produced via the process of intramembranous ossification within the local perichondrium. The bone collar is merely bone tissue appearing as a hollow bone cylinder surrounding the mid-portion of the cartilage model (Junqueira, 1998). This perichondrium ultimately becomes the periosteum (Leeson, 1985). The next step takes place in the diaphysis (central portion of cartilage model) and is degenerative in nature. The characteristics of this step include cell enlargement, matrix calcification and cell death. The result of these processes is a three-dimensional structure formed by the remnants of the calcified cartilage matrix. During this process, blood vessels penetrate through the bone collar and transfer osteoprogenitor cells to this region.

Subsequent to the above process, primary ossification centres form and there is continuous production of primary bone layers around the cartilaginous matrix remnants by the osteoblasts. Secondary ossification centres form at the swellings in the extremities (epiphyses) and together with the primary ossification centre eventually form cavities. These cavities gradually fill with bone marrow. The epiphyseal cartilage is responsible for the growth in length of the bone and when the epiphysis closes, there is cessation in bone growth. Epiphyseal cartilage can be divided into seven zones (Leeson and Leeson, 1981) namely;

- ►the resting zone/reserve zone/quiescent zone
- ►the zone of proliferation

- ►the hypertrophic zone/maturation zone
- ►the zone of calcification
- ►zone of retrogression
- ►the ossification zone
- ►the resorption zone

The resting zone, or otherwise known as the quiescent zone, is mainly composed of hyaline cartilage and is present in the areas nearest to the end of the bone (Leeson and Leeson, 1981). As ossification approaches, this initial short zone progressively shortens and generally shows growth in all directions (Leeson and Leeson, 1981). The next zone (zone of proliferation) is an active zone and involves the cells of the resting zone producing daughter cells, which align themselves in distinct columns parallel to the long axis of the bone. Each row of cells grows by the addition of more cells and so forth. The mechanism (addition of more cells) of this zone allows the cartilage to increase in length (Leeson and Leeson, 1981). The third zone involves the maturation of these cells (hypertrophic/maturation zone) in which large chondrocytes within large lacunae can be found with thin adjoining septae (Junqueira and Carneiro, 2003). The next zone involves the process of calcification of the matrix surrounding these enlarged lacunae, hence the name, zone of calcification (Leeson and Leeson, 1981). Following the calcification, there is a zone of ossification. Here the osteoblasts differentiate from the mesenchymal cells and gather on the exposed plates of calcified cartilage. The osteoblast cells lay down bone at this stage and endochondral bone tissue appears (Junqueira and Carneiro, 2003). The last stage is known as the resorption zone and it is here that the resorption of the bone in the centre of the diaphysis takes place resulting in an increase in size of the marrow cavity (Leeson and Leeson, 1981).

2.1.4. Bone Growth and Remodeling

As bone enlarges, the internal structure continually reconstructs itself and is in a constant state of turnover, otherwise known as remodeling (Stout, 1989). Remodeling is also considered to be the process by which inert mineralized bone is converted to the vital and metabolically active component of an organism (Hall, 2005). On average, cancellous bone at any one surface location undergoes the process of

remodeling every 2 years (Ott, 2002). The process of remodeling requires interactive cellular activity, which is regulated by biochemical and mechanical factors. This process of remodeling helps maintain the shape of the bone during growth (Ott, 2002). Even during adulthood, bone retains the ability to modify its shape and structure in response to environmental changes (Freemont, 1998). The most probable reason for bone undergoing remodeling is to enable the bone to adapt to the mechanical stresses encountered throughout life (Ott, 2002). The simultaneous processes of bone deposition and bone resorption are responsible for maintaining the shape of the bone while it grows. It is these continuous changes that lead to the potential for age determination in adults using bone histology. In young children, the rate at which bone remodels is greater than it is in adults. A variety of factors, including pregnancy, strain on the body, hormones and growth factors may have an effect on the process of bone remodeling.

During the initial deposition of the lamellar bone, small blood vessels are incorporated into the lamellar bone. These vessels can potentially become surrounded by concentric lamellae. When this occurs, these canals are known as primary osteons (Robling and Stout, 2000) (Figure 2.1). If the vessels do not have any lamellae surrounding the periphery of the canal, they are simply termed non-haversian canals (Robling and Stout, 2002). These primary canals are distinguishable from Haversian systems (Figure 2.2) by their lack or small amount of concentric lamellae (Figure 2.1). The central canal of a primary osteon is approximately 100 μm in diameter and lacks the characteristic cement line observed in secondary osteons (Hall, 2005).

Before Kerley's research, non-haversian canals were generally not recognized as a separate canal type (Enlow, 1963). It was, however, realized that these canals did not form part of the Haversian system and thus should be properly termed. The process of remodeling is not involved in the formation of a primary osteon. The primary osteon is usually relatively small and can contain up to three rings of lamellae. They are seldom large structures. Primary osteons commonly organize in distinct rows or layers and are more widespread through a young, rapidly growing skeleton (Enlow, 1963).

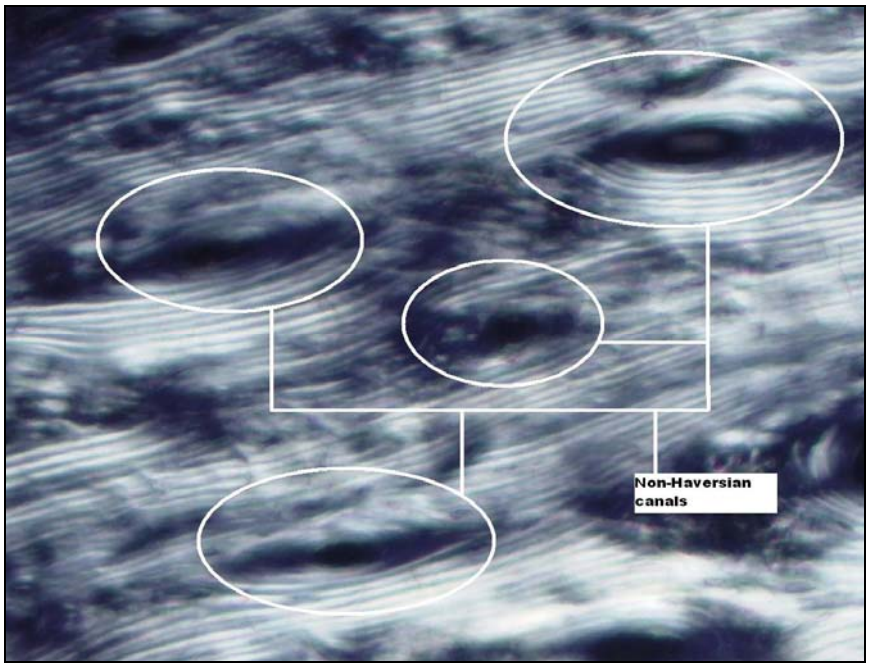


Figure 2.1 Illustration of non-haversian canals situated within lamellar bone

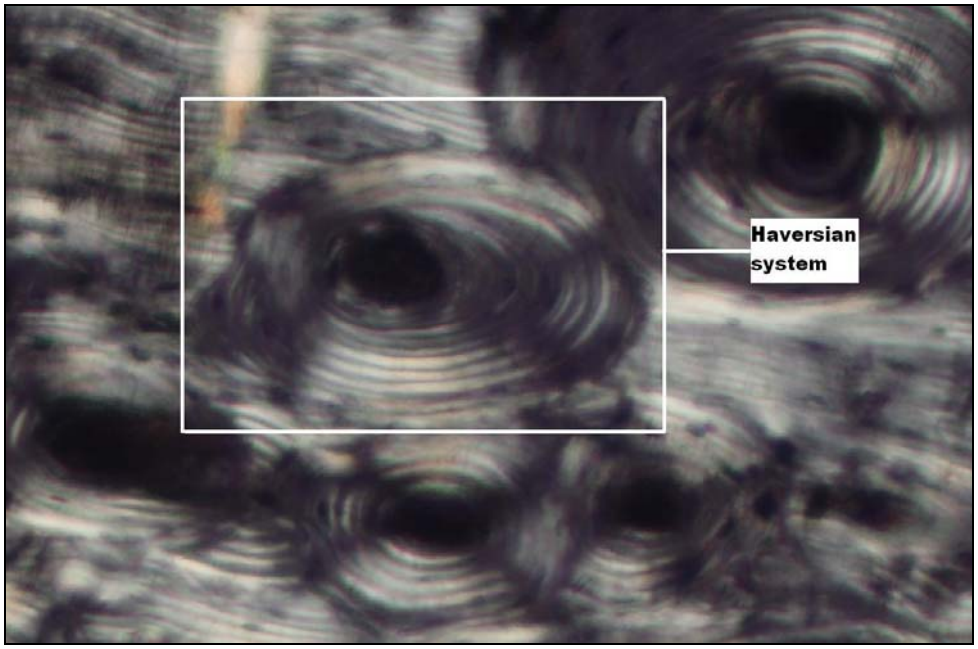


Figure 2.2 Illustration of a Haversian system (secondary osteon)

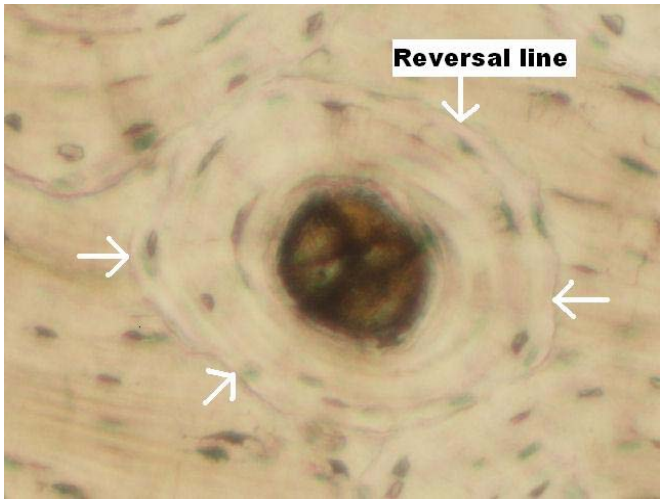
2.1.4.1. Haversian System Development (secondary osteon)

Compact bone, due to its vascular nature in most vertebrates, is a network of canals through which various vessels pass. Canals can be either primary or secondary and are capable of branching in a variety of characteristic directions (Enlow, 1963).

The term osteon is defined as an elongated cylinder with a canal that is surrounded by concentric layers of osteocyte-bearing lamellae (Enlow, 1963). It is also known as the basic structural element of mineralized cortical bone (Hall, 2005). An osteon that is found superimposed on previously existing primary bone is termed a secondary osteon. The first step in the formation of Haversian systems is the enzymatic activity of the osteoclasts. Osteoclasts drill a cylindrical hollow (tunnel/resorption cavity) through the compact bone, usually vertical to the long axis of the bone. The osteoclasts resorb the primary bone at a rate of about 40 – 50 µm per day (Scheuer and Black, 2000). Blood vessels, nerves and lymphatic vessels occupy this hollow. The borders of these resorption cavities are highly distinguishable by scalloped edges that indicate the presence of Howship's lacunae (Stout, 1989). When the tunnel is large enough (± 250 - 300µm), osteoblasts surround the inner circumference and almost immediately start to deposit bone matrix in successive, concentric lamellae at a rate of 1 – 2 µm per day (Robling and Stout, 2000; Scheuer and Black, 2000). In addition, the osteoblasts lay down a thin layer of cement, otherwise known as a reversal line (Figure 2.3). This reversal line marks the original face of the resorption canal (Maat, 2003) and is characterized as a thin, mineral-deficient, sulphur-rich layer of matrix (Schaffler *et al.*, 1987).

The osteoblasts deposit the bone matrix from the periphery of the newly formed resorption space, inward, thus narrowing the final diameter of the Haversian canal. Once the osteoblasts have surrounded themselves with bone matrix, they are known as osteocytes (mature osteoblasts). During this process, the cytoplasmic processes of the osteocytes are laid down in the matrix, which forms the communication system (canaliculi) between the cells. At a specified point, deposition of the lamellae ceases and a Haversian canal is left in the centre of the system (Robling and Stout, 2000). As remodeling occurs, portions of old lamellae are left inbetween the newly formed osteons and these portions of lamellae are known as interstitial lamellae (Stini, 1995).

a)



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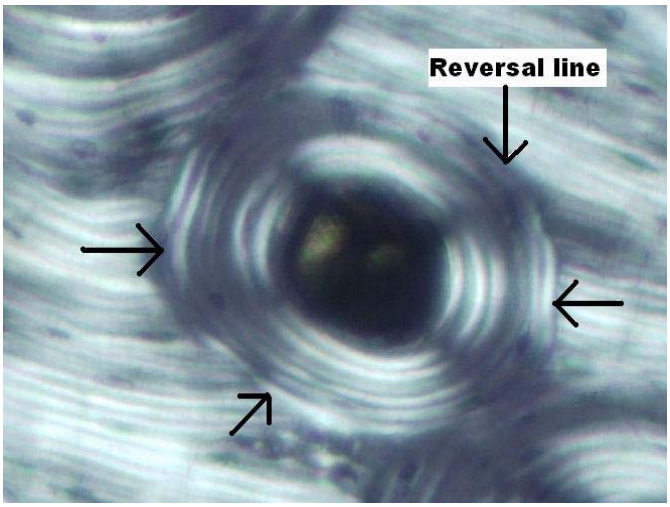


Figure 2.3 Illustration of a reversal line in the same Haversian system as seen in a) Transmission light microscope and with b) polarized lens

With time the bone matrix in each of the lamellae becomes mineralized. The lamellar bone remaining between the osteons is known as interstitial lamellar bone. Volkmann's canals are found, usually at right angles, between osteons and carry the blood vessels, nerves and lymphatic vessels between the osteon canals.

Various types of secondary osteons are found throughout compact bone. Type II osteons can be distinguished by their position in the bone (Figure 2.4). These osteons are otherwise known as embedded osteons, even though they contain the complete reversal line and concentric lamellae (Robling and Stout, 2000). These osteons form within other osteons without crossing the reversal line (cement) of the preexisting osteon (Robling and Stout, 1999).

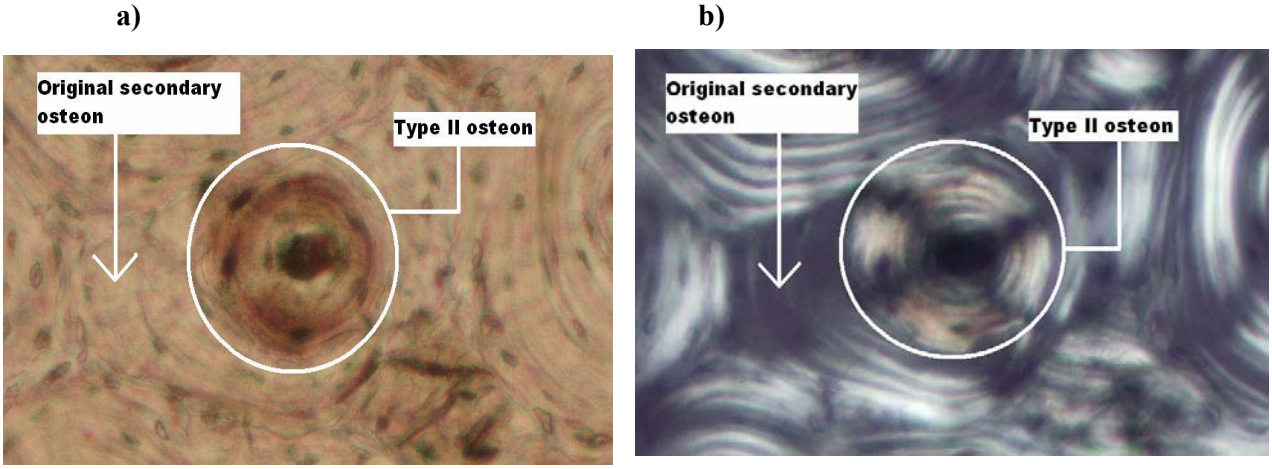
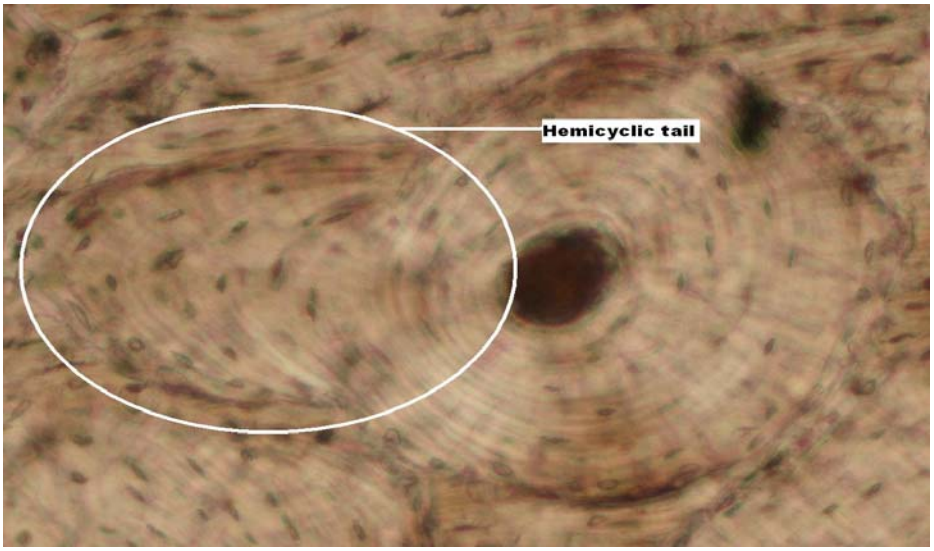


Figure 2.4 Illustration of the same Type II osteon seen with a) Transmission light microscopy and b) a polarized lens

Type II osteons are formed by the radial remodeling of a pre-existing Haversian canal and appear to be completely embedded in a larger osteon (Robling and Stout, 2000). Double-zoned osteons can be distinguished from the other osteons by the presence of a hypercalcified ring within the lamellae and the lack of an internal reversal line (Robling and Stout 2000; Ericksen 1991).

Robling and Stout (1999 & 2000) also defined a third type of osteon: drifting osteon: which occurs when the group of cells responsible for the remodeling of bone (BMU: basic multicellular units) travels longitudinally and transversely through the cortex resulting in an elongated osteon displaying a hemicyclic tail (Figure 2.5). It is defined as an osteon that is in a state of resorption on one side and continuous bone formation on the other side (Robling and Stout, 1999).

a)



b)

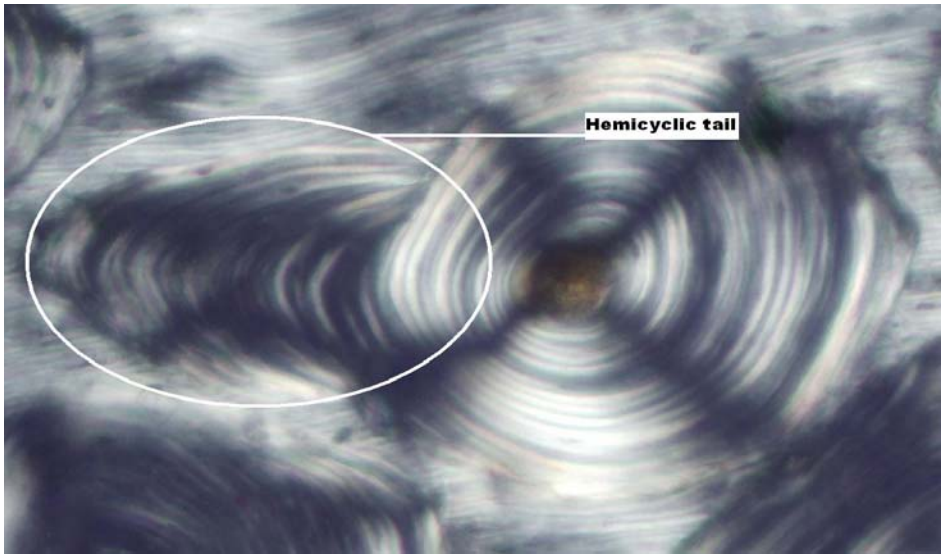


Figure 2.5 Illustration of the same hemicyclic tail seen with a) Transmission light microscopy and with b) a polarized lens

2.1.5. Factors that influence bone formation and maintenance

Many factors play a role in maintaining and the ossification of bone. Most of these factors (intrinsic and extrinsic) have a drastic effect on bone formation to the extent that it could affect the age estimation process.

2.1.5.1. Disease

Many diseases have a profound effect on the formation of bone that may permanently affect the microstructure. Most of these diseases affect sections of the entire skeleton and not just one or two isolated bones.

Paget's disease

Paget's disease is a focal disorder that chronically affects the turnover rate of bone (Selby, 2002) resulting in skeletal, vascular and articular complications (Eekhoff *et al.*, 2003). The trademark of this disease is the excessive and disorganized activity of bone (Smith, 1979). The disease involves the rapid resorption of bone and consequently the rapid deposition of new bone, with the histological sections displaying increased numbers of osteoblasts and osteoclasts (Smith, 1979) and having a mosaic-like pattern of Haversian systems (Ortner, 1981). Due to the increase in bone formation there is an increased percentage of osteoid on the surface of the affected bones (Smith, 1979). This disease can lead to deformities in the skull, spine and long bones (Tucci, 2004) with an increased thickness of the bone surface (Smith, 1979).

The incidence of Paget's disease is unknown (Tucci, 2004) and the cause remains uncertain. Siris (1996) suggests that the affected individuals may be genetically predisposed, with the osteoclasts and their precursors being affected by a paramyxovirus infection (Siris, 1998). The disease is found to be more predominant in Northern European populations (Selby, 2002), less prevalent in African blacks and Scandinavians, and rare in Orientals and Asian Indians (Tucci, 2004). Paget's disease is also more prevalent in individuals over the age of 40 (Ortner, 1981).

Osteoporosis

Osteoporosis is a disease that is caused by the imbalance of the processes of bone formation and bone resorption (Smith, 1979), leading to an increased risk of fractures (Stini, 1995). It was previously believed that the rate of formation slowed down as the rate of resorption increased, but it is now known that the rate of formation remains relatively normal while the rate of resorption increases drastically (Jowsey, 1977). This increase in resorption results in the characteristic loss of bone mass seen with osteoporosis. The disease is associated with increasing age and is therefore seen more often in the elderly. Fractures associated with osteoporosis are more likely to occur in European populations than among Asian or African groups (Stini, 1995).

The most common occurrence of osteoporosis is in postmenopausal women, and is, to some extent, due to an estrogen deficiency, with the disease affecting most frequently the spine and the femoral neck (Jowsey, 1977; Stini *et al.*, 1992 Westmascott, 1995). Osteoporosis is also more likely to affect white females than black females, which is possibly due to the fact that people of African origin reach skeletal maturity with much more bone mass than what is seen in persons of European decent (Jowsey, 1977).

Hormonal imbalances

Hormones play an intricate role in the maintenance of the intracellular matrices as well as in changes during the ageing process (Little, 1973). Pathological changes in hormone balance may be seen directly on the bone, as in osteoporosis and hyperparathyroidism (Little, 1973). Osteon based age-estimations are only affected by chronic conditions and long-standing metabolic diseases and not by acute conditions, which have no effect on the long-term nature of bone and the process of remodeling (Stout, 1998). Hyperparathyroidism is the excess release of the parathyroid hormone and is known to have no effect on the modelling of bone but only an effect in the activation frequency (Robling and Stout, 2000). Even though this is true individuals that are suffering from hyperparathyroidism seem to have a greater amount of osteons if compared to unaffected individuals thus influencing histological ageing techniques (Robling and Stout, 2000). This indicates that although hyperparathyroidism has no effect on the modeling process of bone, the hormone imbalance must have an effect on the remodeling process for there to be a difference in the number of osteons as the authors indicate. This is particularly

relevant to extinct populations and a few existing populations where there were or are many dietary stresses (Robling and Stout, 2000). Dietary stresses occur when there are inadequate amounts of food and nutritional sources such as protein. Calcium and carbohydrate deficiencies can also contribute to dietary stresses. A deficiency of calcium particularly, is known to induce the excessive release of the parathyroid hormone, which triggers the increased differentiation of the osteoclasts, and results in the above-mentioned disease (Robling and Stout, 2000).

Metabolic and nutritional diseases

Certain diseases that affect the complex nature of bone can be attributed to a variety of nutritional deficiencies. Of the diseases that affect bone, scurvy, rickets and iron deficiency anaemia are best documented. Scurvy is the result of a vitamin C deficiency that causes a reduction in osteoid production, weakness in connective tissue and hemorrhagic diathesis (Stuart-Macadam, 1989). The general manifestations seen in the bone is subperiosteal haemorrhages (more in infants) as well as, reduced bone formation with normal resorption activity. In adults there is a slight thinning of the cortical bone from the formation of new periosteal bone as the result of the subperiosteal haemorrhaging (Stuart-Macadam, 1989). With the healing of this disease, the new periosteal bone that has formed, settles onto the shaft of the bone resulting in the consequent thickening of the cortex (Stuart-Macadam, 1989).

Rickets is a disease that is only found in young individuals and is the result of a vitamin D deficiency. The manifestations of this disease include bone deformation under pressure as a result of the poor mineralization of newly formed bone (Stuart-Macadam, 1989), and resulting in epiphyseal plate distortion due to the natural body strains (Junqueira and Carneiro, 2003). Children with a deficiency of calcium may also develop rickets. Adults, who present with a calcium deficiency, may develop osteomalacia, which is the poor calcification of new bone and the partial resorption of existing calcified bone (Junqueira and Carneiro, 2003).

Paine and Barrett (2006) established that there is a definite influence of nutritional diseases on histological ageing techniques, specifically the method developed by Stout and Paine (1992). The results of this study showed that when the regression formulae developed by Stout and Paine (1992) to determine age at death were applied to individuals who had died from a nutritionally related disease or pellagra, the age of these individuals was underestimated on average by 29.2 years. They also noted that

the size of the secondary osteons and the Haversian canals were larger while the cortical bone area was diminished in the individuals that had suffered from malnutrition or pellagra.

2.1.5.2. Population variation

When Kerley (1965) first presented his research on age estimating of bone from its microscopic structures, the possible differences between population groups were not considered. Most of the research that followed applied the same thoughts and did not determine whether there were any possible differences in the microscopic structures of bone between various population groups. Although investigations into the differences seen between populations groups were being conducted in the 60's, 70's and 80's (eg., Moldawer *et al*., 1965, Nordin, 1966, Ericksen, 1976, Ruff and Hayes, 1982, 1988, Evers, *et al.*, 1985, Martin *et al*., 1985), few researchers applied them to microscopic ageing techniques. The most common reason was probably the lack of a diverse sample. Various authors have tested Kerley's method on a variety of populations with results ranging from 42% giving the correct age within ± 5 years (Fangwu, 1983), to 70% of the age estimates being underestimates (Aiello and Molleson, 1993).

Thompson (1979) indicates that the application of the same regression formulae to various population groups requires a sufficient sample of known age and known population. Ericksen (1991) made use of a large heterogeneous sample in the hope of making the method more significant to a variety of populations across the USA. Cho *et al.* (2002) conducted a population-specific study using African-American and European-American skeletal remains. The results obtained from the Cho *et al.* (2002) study indicated that there is a definite difference in the osteon population density, the osteon area and the relative cortical area of the bone between the populations, which must be considered when developing or using a histological technique for age determination. Recently Qiu *et al.* (2006) looked at the differences between the osteocyte and lacunar densities in the iliac bone of black and white American females. Thirty four black women (24 – 70 years) and 94 white women (20 – 73 years) that were skeletally healthy, and had had previous bone biopsies performed were used for this study. The results indicate that there seems to be more osteocytes in black female individuals than in white females. This conclusion signifies that black individuals have less bone turnover than whites, which adds to the seemingly higher bone mass,

resulting in increased bone strength. Although these differences were found between black and white females, the authors state that these differences are not related to age and may be attributed to other factors like socio-economic status, nutritional status or effects of mechanical stresses (work related) (Qiu *et al.*, 2006). Since the majority of the authors have not fully explained the background histories of the samples, it is difficult to accept that the only reason for the variations seen in the bone histology is biological in nature.

2.1.5.3. Sex

It has been established that older men and women exhibit different rates of bone remodeling, but it is unknown whether there are different rates of remodeling in younger men and women (Robling and Stout, 2000). Thompson (1980) found that females have larger Haversian canals in the femoral midshaft than males, but males seem to have more canals in general. Thompson (1980), however, suggests that this difference may be due to sampling distribution (Burr *et al.*, 1990). The question remains whether this difference in rates of remodeling of the older individuals has a significant effect on the accuracy of histological techniques in determining age (Robling and Stout, 2000). Kerley (1965) found that sex did not have a significant role when estimating age and many of the researchers that followed (Ahlqvist and Damsten, 1969; Singh and Gunberg, 1970) concurred with that conclusion.

Thompson (1979) and Ericksen (1991) both separated the regression formulae to incorporate sex-specific equations. Ericksen found that the sex-specific equations yielded better results than the combined equations. She found that there were significant differences in relation to the number of osteons and the number of osteon fragments. Pfeiffer (1998) found no significant sex differences in the size of osteons in 20th century South Africans, 19th century Canadians and 18th century English settlers. This finding is contradictory to the findings of Burr *et al.* (1990), who indicates that the osteons in females increase in size with age while the osteons in males seem to decrease in size with age. Another factor that could play a fundamental role in the differences seen between males and females is the skeletal maturation and timing of growth cessation. It is well known that epiphyseal closure in males and females varies and this could account for the differences seen between males and females, particularly in younger individuals.

A more common factor playing a role in the difference between the bone structure of male and female individuals is microdamage. Microdamage is the result of continuous strain on the skeletal system throughout everyday life. Norman and Wang (1997) concluded in their research that microdamage of bone is significantly greater in females, and that microdamage is more prevalent in the midshaft of the femur and tibia than in other bones. The relevance of this to histological ageing techniques is that microdamage promotes an increase in remodeling and is known to be significantly age and sex dependent (Norman and Wang, 1997), thus influencing the appearance of bone structures.

The degree of mineralization of the human femoral midshaft has been shown to display significant differences between males and females (Goldman *et al.*, 2003). Goldman *et al.* (2003) found that there is a decrease in the overall degree of mineralization with age but an increase in the coefficient of variation. The coefficient of variation is a useful application for standardization purposes and explains the variation of a variable in relation to the mean (Allan, 1982). Males between the ages of 45 – 65 years tend to have a higher degree of mineralization than females of the same group. It seems to be the opposite case in older individuals (65+) where there appears to be a decrease in the degree of mineralization in older males but not in females. Low mineralization is seen in areas of high bone turnover when compared to areas of low turnover. According to Goldman *et al.* (2003), males tend to show a higher rate of bone turnover than females do, thus male individuals have more frequent and more efficient renewal of bone. The authors (Goldman *et al.*, 2003) believe that this variability in the degree of mineralization is an important factor to consider when trying to understand the biomechanical adaptation of bone and its age related changes.

A recent study conducted by Maat *et al.* (2006) indicates that there is no statistically significant difference between males and females with respect to the percentage of unremodeled bone when dealing with a Dutch sample. Cho *et al.* (2006) conducted a study to establish the possible sex and population differences in the patterns of age-associated bone loss. The results indicate that African American females tend to loose more bone when compared with the African American male but this difference is not observed amongst the European American sample (Cho *et al.*, 2006).

2.2. Brief history on histological age at death determination techniques

In 1960, Jowsey studied the microscopic appearance of normal bone and its relationship with age. A cross-section of the femur was used. Modeling and resorption are the most important processes in bone formation and it was found that these two activities appear to take place only on the surface of the bone (Jowsey, 1960). Bone formation, according to Jowsey, can be distinguished by the presence of low mineral density lamellae that run parallel to the central canal of the osteon and bone resorption could be classified as an uneven surface of high mineral density with the lamellae running at any angle to the surface. The process of osteoid deposition takes place during bone formation. When examining a section of bone, osteons will be seen at different degrees of mineralization due to this process taking place over different periods.

The results of Jowsey's study indicated that in young adolescent individuals there is a high percentage of bone formation and bone resorption, thus implicating a high rate of bone turnover. There is also a high porosity present in young individuals due to the large number of forming osteons and a high rate of bone resorption. When the results of the young adults were examined, it is seen that the bone turnover rate is drastically reduced and there is little bone formation and bone resorption. The bone also displays high mineral density giving the bone a very compact appearance. Older individuals display a gradual increase in resorption, but not in formation.

In 1965, Ellis R. Kerley established the first method of age at death determination of skeletal remains, using a histological technique. In the study, 126 ground bone cross-sections were taken from the midshaft of the femur, tibia and fibula. Of the sample, 88 were male, 29 were female and nine had no sex recorded. The recorded ages of the sample ranged from birth, right through to 95 years and included an individual from every decade. Any bones showing signs of pathology were excluded.

Four circular fields were chosen as the areas of examination and the fields were viewed on the outer third of the cortex, as that is where the observed chronological series of structures seemed to be the most age-specific (Figure 2.6).

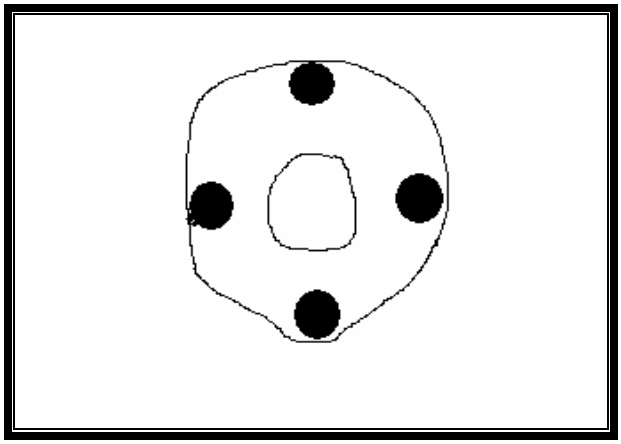


Figure 2.6 Diagram of a cross-section through a long bone, illustrating the location of the four view fields

Four microstructures were counted:

1. The total number of osteons in all four fields
2. The total number of old Haversian fragments
3. The percentage of circumferential lamellar bone
4. The total number of non-haversian canals

Kerley described the osteons as being vascular canals surrounded by concentric lamellae, which contain rather evenly spaced osteocytes. Fragments were considered the result of osteoclastic activity through the process of remodeling. Circumferential bone is composed of evenly spaced bands that run parallel to each other around the outer cortex and is highly prominent in young individuals. The non-haversian canals include all primary vascular channels, including those that are partly filled with concentric lamellae to form primary osteons.

Kerley examined the sections under a 100-x magnification wide-field microscope and a field diameter of 1.25 mm which was later revised by Kerley and Ubelaker to 1.62 mm (Kerley and Ubelaker, 1978). In the revised paper, Kerley and Ubelaker developed a formulation to ensure that the field size remains constant. With the use of a stage micrometer, the area of a 100-x field should be calculated and then divided into 2.06 mm^2. This would establish a relationship between the original field size and

the individual size. With the factor obtained, all counts, excluding the percentage of circumferential lamellar bone, must be multiplied by it.

Kerley's results indicated that there is an increase in the number of osteons between birth and adulthood. It was also found that the number of osteon fragments increased with age. The percentage of circumferential bone, however, decreased with age as well as the number of non-haversian canals. Kerley noted that after the age of 55, the appearance of non-haversian canals actually ceased altogether. The data obtained was subjected to linear and curvilinear regression analysis and all criteria used showed a significant correlation with age with the most significant being the fibular osteon fragments (Table 2.1). This factor was so accurate that the regression for the fibula could be used alone and the calculated age will be within ± 10 years of the actual age 95% of the time.

Table 2.1 Regression formula (Kerley and Ubelaker, 1978) for calculating age at death (Y) from cortical microstructure with *S: square root of the mean square residual. (X = value of the individual factors in column one)

Factor	Regression formulae	*S
Femur		
Femoral osteons	$Y = 2.278 + 0.187X + 0.00226X^2$	9.19
Femoral fragments	$Y = 5.241 + 0.509X + 0.017X^2 - 0.00015X^3$	6.98
Femoral lamellar (%)	$Y = 75.017 - 1.790X + 0.0114X^2$	12.52
Femoral non-haversian	$Y = 58.390 - 3.184X + 0.0628X^2 - 0.00036X^3$	12.12
Tibia		
Tibial osteons	$Y = -13.4218 + 0.660X$	10.53
Tibial fragments	$Y = -26.997 + 2.501X - 0.014X^2$	8.42
Tibial lamellar (%)	$Y = 80.934 - 2.281X + 0.019X^2$	14.28
Tibial non-haversian	$Y = 67.872 - 9.070X + 0.440X^2 - 0.0062X^3$	10.19
Fibula		
Fibular osteons	$Y = -23.59 + 0.74511X$	8.33
Fibular fragments	$Y = -9.89 + 1.064X$	3.66
Fibular lamellar (%)	$Y = 124.09 - 10.92X + 0.3723X^2 - 0.00412X^3$	10.74
Fibular non-haversian	$Y = 62.33 - 9.776X + 0.5502X^2 - 0.00704X^3$	14.62

Following Kerley's research in this field, the next 40 years yielded various other methods. The methods that followed Kerley's, except for the one developed by Ahlqvist and Damsten, focused on a variety of microstructures bone present in bone.

Ahlqvist and Damsten (1969), instead, focused purely on percentage of structures increasing with age (osteons and osteon fragments) and those that decrease with age (lamellar bone and non-haversian canals).

The authors' aim was to simplify Kerley's method and to reduce all bias caused by having to identify structures to the best of one's ability. Twenty ground sections were used to test this altered method. An ocular square-ruled network consisting of 100 squares was superimposed on the 20 ground sections and instead of using a circular view like Kerley, Ahlqvist and Damsten used a square field. They also used different locations on the outer cortex of bone (Figure 2.7).

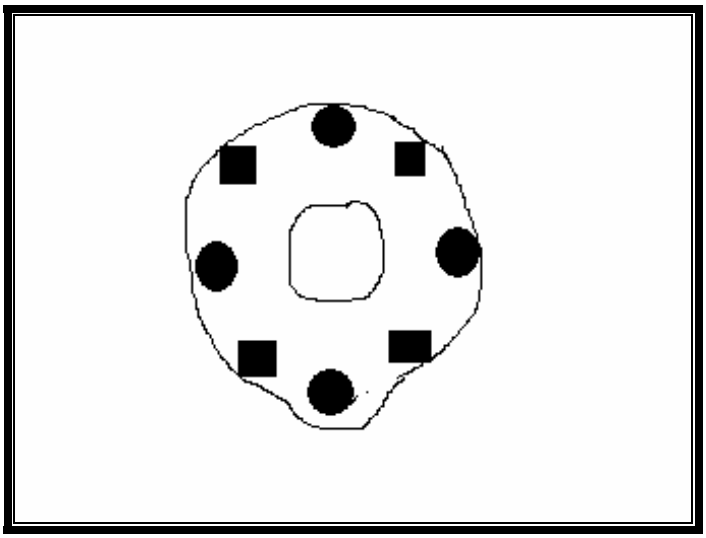


Figure 2.7 Diagram illustrating the square fields used by Ahlqvist and Damsten compared to the circular fields used by Kerley.

This type of square-field analysis aids in identifying those structures which are on the periphery of the 100 square grid so as not to get confused as to what structure lies on the outside of the field. The squares were analyzed and counted; the count was dependent on which structures were more than 50 percent dominant in the square. The results of this new method showed a slight inferiority to those results obtained by Kerley. The percentage of femoral osteons and osteon fragments yielded a standard error of estimate (S.E.E.) of ± 6.71 years and when compared to the S.E.E. of ± 5.27 years obtained from Kerley's fibular fragments, it is clear that Kerley's method was

more accurate. Ahlqvist and Damsten, however, stated that their altered method yielded improved results when other singular factors were taken into account.

The main problems, encountered by Ahlqvist and Damsten when trying to repeat Kerley's method, were in distinguishing between an osteon and an osteon fragment and in estimating the percentage of circumferential lamellar bone.

Singh and Gunberg (1970) developed their method of age at death estimation from the microscopic structure of bone that mainly concentrated on estimating age in a much older human population. A sample of 59 (52 male and 7 female) cadavers aged from 39 to 87 years was used, and a 1cm x 1cm fragment of the midshaft of the anterior surface of the femur and tibia was removed. Samples were also taken from the posterior border of the mandibular ramus. Out of the 59 individuals, only 40 (33 male) had all three bones present, while the remaining 19 (all male) only had the mandible available. The female sample was small, resulting in the fact that the models for age estimation were derived exclusively for males. The female sample was used to test the model and to ascertain any sex differences.

The samples were prepared and mounted onto glass slides where they were viewed under a light microscope for examination. A 10x objective combined with a 10x widefield ocular lens permitted a view field of 2 mm in diameter. Two view fields were chosen randomly along the outer third and close to the periosteal edge of the bone. Singh and Gunberg (1970) chose three distinctive characteristics from the bone to use for this method, namely:

1. The total osteon count (X1)
2. The average concentric lamellae per osteon (X2)
3. The average Haversian canal diameter (X3 - smallest diameter per canal)

They did not include non-haversian canals or osteon fragments in their observation criteria, the motive being that non-haversian canals are less likely to be visible in individuals over the age of 55 years (Kerley, 1965) and the sample consisted purely of individuals over the age of 40. Singh and Gunberg excluded osteon fragments for two reasons: one, the interstitial tissue normally consists of fragments and two; osteons are not always parallel to the axis of the long bone and could therefore be cut obliquely giving the appearance of an apparent osteon fragment, thus resulting in possible errors in counting.

Once the data was obtained, it was first subjected to simple correlations for each independent variable and then multiple linear regression analysis. Formulae for each of the femur, tibia and mandible were established (Table 2.2).

Table 2.2 Regression equations for estimating age in humans from histological measurements (Singh and Gunberg, 1970) (dependent variable: age in years) (X1 = total osteon count, X2 = average lamellae/osteon and X3 = average Haversian canal diameter)

Selection	Regression equations	Multiple R1	Std. error of estimate
Mandible			
I	20.82 + 0.85X1 + 0.87X2 - 0.22X3	0.979	2.55
II	-18.99 + 1.13X1+ 1.76X2	0.976	2.69
III	32.23 + 0.92X1 - 0.30X3	0.978	2.58
IV	74.73 + 1.52X2 - 0.45X3	0.969	3.04
V	-28.24 + 1.68X1	0.969	3.02
VI	5.31 + 5.00X2	0.950	3.83
VII	103.99 - 0.63X3	0.966	3.16
Femur			
I	27.65 + 0.65X1 + 0.78X2 - 0.26X3	0.958	3.24
II	-14.69 + 1.13X1 + 1.11X2	0.948	3.55
III	29.59 + 0.79X1 - 0.28X3	0.957	3.25
IV	61.25 + 1.74X2 - 0.44X3	0.949	3.52
V	16.10 + 1.38X1	0.945	3.60
VI	2.00 + 5.16X2	0.889	5.01
VII	89.01 - 0.62X3	0.937	3.82
Tibia			
I	43.52 + 0.291X1 + 1.47X2 - 0.34X3	0.964	3.02
II	-3.40 + 0.67X1 + 2.27X2	0.936	3.93
III	48.61 + 0.53X1 - 0.38X3	0.957	3.22
IV	54.79 + 2.19X2 - 0.4X3	0.960	3.12
V	-4.76 + 1.15X1	0.919	4.33
VI	5.10 + 4.88X2	0.908	4.59
VII	91.32 - 0.64X3	0.935	3.88

Although the three parameters showed significant correlation with age, there were variations. The largest variation was seen in the average number of lamellae per osteon and the average Haversian canal diameter and the least variation was observed in the total number of osteons. The simple correlation analysis showed that the total number of osteons and the Haversian canal diameter, demonstrated better correlation with age than the number of lamellae per osteon. The authors also found that the mandibular correlations were slightly higher than that of the correlations of the femur and the tibia.

Due to the limitations of the sample size, a nomograph was constructed from the mandibular measurements that provided a multiple correlation of 0.978 with a standard error of 2.58 years. The results demonstrate that between the ages of 40 and 80 years, age could be accurately estimated within ± 2.58 years in 67% and within ± 5.16 years in 95% of the individuals. This investigation established that there is a significant age-associated change in the Haversian canal diameter which contradicts the earlier findings of Currey. Currey (1964) stated that there is little or no age-associated change in the Haversian canal diameter. Singh and Gunberg worked in accordance with Kerley, specifying that there is no significant difference between the sexes, even though both authors only had a small female sample group. No mention of differences in racial affinity is mentioned in Kerley's paper but Singh and Gunberg state that the data regarding racial distribution in the sample was limited. The authors furthermore indicate that future studies should include the possible differences observed in individuals due to sex or population affinity.

In 1979, Thompson established the first histological ageing technique that did not require sampling a full cross-section of the bone. The advantage of applying this technique is the reusability of the bones afterwards as they are not destroyed. Thompson's sample consisted of 116 individuals (64 male and 52 female) which were all over the age of 29 (mean age 71.48 years). Cores with a diameter of 0.4 cm were extracted from the anterior midshaft of the femur, the medial midshaft of the tibia, the midshaft of the humerus medial to the deltoid tuberosity and one third of the distance from the distal end of the ulna (lateral surface). The samples were removed using a corer attached to a high-speed dremel drill. Nineteen variables were chosen and these included histological characteristics as well as other variables that are not derived histologically, for example cortical thickness or bone density. The bone sections were examined using a phase-contrast microscope at x100.

All results were subjected to linear regression analysis and Thompson found that the osteon area showed the most consistency when estimating age at death and was to a degree the most accurate. The results also indicated that the lowest SEE (standard error of estimate) was 6.21 years and this was obtained from the humerus. This study also revealed that after the age of 50 there is a constant degradation of the cortical thickness and bone mineral density with age, with the females losing 8% cortical thickness and 10% bone mineral density per decade and males losing only 4% cortical thickness and 6% bone mineral density per decade. Thompson tested this method on

eight forensic cases and was accurately able to determine the age at death with the highest difference being ±5 years. Pfeiffer (1992) tested Thompson's method on a sample of 29 adult skeletal remains from 19th century historic cemeteries. She found that only eight of the 16 known individuals had accurate age estimates, while 5 were overestimated and 3 were underestimated. The study done by Pfeiffer concluded that the Thompson method was relatively robust and required more development.

In 1991, Ericksen designed a method of determining age at death solely from the histological structure of the femur. A heterogeneous sample of 328 individuals (154 female and 174 male) was used in the study. The purpose of using such a diverse sample was to attempt to make the results widely applicable. The age range of this sample was 14 – 97 years with a mean age of 62.76 years. Pathology was not considered as an exclusion factor in this study for two reasons; one, that the reporting of bone-affecting diseases was unreliable and two, age estimating methods are supposed to be used on individuals with an unknown medical background. Therefore to exclude for pathology would bias the results.

The sampling method that Ericksen applied was semi-destructive in nature and involved removing a small 1.0 cm slab from the anterior midshaft of the femur, opposite the linea aspera. The bone sections were ground down to approximately 100μ and mounted onto slides. Five points were marked out on the section, one in the centre, two 0.5 cm on either side of the centre mark and another two 0.25 cm from the centre mark. These marks indicated where the five view fields were allocated. The sections were examined at a magnification of approximately x 32.5 using an Olympus camera-mounted microscope and an Olympus PM-CBA control unit. A rectangular photographic field (0.886 mm^2) was obtained. For the five fields viewed per section in this study the total area amounted to 4.43 mm^2.

The following observations were made from the sections:

1. The secondary osteon count (X_1).
2. The type II osteons (X_2). According to Ericksen, these contain two cement lines that do not touch one another.
3. The osteon fragments (X_3).
4. Resorption spaces (X_4).
5. Non-haversian canals (X_5).

6. Average percent circumferential lamellar bone (X_6).
7. Average percent osteonal bone (X_7).
8. Average percent fragmental bone (X_8).

The data obtained were subjected to linear regression analysis and multiple regression analysis to determine the correlation with age. Ericksen also conducted a brief examination of the possible factors responsible for any sex differences observed during the regression analysis. The results obtained from this study indicate that all variables except for the resorption spaces show a statistically significant correlation with age. Ericksen also determined sex-specific equations. Twenty-eight regression equations were calculated in total. Ericksen found only three equations to work efficiently in practice (Table 2.3).

Table 2.3 Selected regression equations found to be the most suitable by Ericksen from work practice (Ericksen, 1991). S.E.E. = Standard error of estimate (in years)

Regression equation	r^2	S.E.E.
Sexes combined (N = 328)		
8. Y = 67.43 + 1.11X1 + 2.46X2 + 0.20X3 - 1.57X5 - 0.30X6 - 0.39X7	0.67	10.08
Females (N = 154)		
18. Y = 63.39 + 0.55X1 + 3.12X2 + 0.20X3 + 0.92X4 - 1.57X5 - 0.31X6 - 0.24X7	0.71	10.00
Males (N = 174)		
26. Y = 57.98 + 1.36X1 + 1.90X2 + 0.32X3 - 1.62X5 - 0.17X6 - 0.33X7	0.64	10.05

The lowest SEE obtained in Ericksen's study was 9.96 years, which was for females when all eight variables were used in the regression equation. The highest SEE is seen in all cases where just one or two variables were used to derive the equation. Ericksen found that the difference between the sexes was only significant in two aspects of the bone histology, namely the number of osteons and the number of osteon fragments.

Stout and Paine (1992) introduced a technique for determining age at death from the histological structure of the rib and clavicle. A sample of 40 individuals (32 males, 7 female and 1 unknown sex) of which 32 were white, 4 black and 4 unknown was used. The ages ranged from 13 – 62 years (mean age = 28.6 years). The authors furthermore had access to an additional 12 ribs and 7 clavicles obtained from autopsies and forensic cases that were used to test the method.

The samples were viewed using a standard research microscope with x16 and x20 objectives. A 10x ocular lens fitted with a standard reticule was used for microscopic analysis. The following measurements were included:

1. Cortical area
2. Intact osteon density
3. Fragmentary osteon density
4. Total visible osteon density

All results were subjected to regression analysis and age-predicting formulae were developed. The use of the clavicle and ribs can be useful in determining age at death as it utilizes the bone from the torso. This helps in cases where no long bones are available to use. The formulae developed by the authors are best utilized when both the rib and clavicle are available. In 1996, Stout *et al.* reviewed this method with the purpose of testing it and making corrections if any were needed. A sample of 83 adults (41 male and 41 female) was used to test the method. The results indicated a mean absolute difference of 5.5 years between the known ages and the estimated ages with none of the predicted ages falling outside the confidence interval of 95%.

Following this method, Stout *et al.* (1994) developed another method of determining age at death using just the sternal end of the fourth rib. A sample of 60 sternal rib ends were obtained and the individuals' ages ranged from 11 – 88 years (mean age = 39.2 years). A transverse cross-section was removed and slides prepared. The same method mentioned above (Stout and Paine, 1992) was employed to perform the histological analysis of the sections and the following histomorphometrics were determined:

1. Intact osteon density (P_i)
2. Fragmentary osteon density (P_f)
3. Osteon population density (OPD)

The osteon population density was used by the authors as an independent variable for the regression analysis. No statistical significant difference was found between the sexes, although the authors never mentioned the total number of males and females making up the sample of 60.

Yoshino *et al.* (1994) conducted an investigation using the humerus to develop a method of age at death determination. Forty males, ages ranging from 23 – 80 years (mean age = 47.6 years), were used in the study. No females were included although the authors recommend that in future research, age predicting equations for females should be developed. The authors made use of the anterior half of the humerus near the surgical neck and a cross-section was taken and ground down to approximately 100µm. Microradiograms of the sections were taken using an OMC-603 soft X-ray apparatus. The following parameters were observed:

1. Secondary osteon number
2. Double-zoned osteon number
3. Type II osteon number
4. Low-density osteon number
5. Osteon fragment number
6. Resorption space number
7. Total osteon area
8. Total Haversian canal area
9. Average osteon area
10. Average Haversian canal area

The data obtained from the analysis was once again subjected to linear and stepwise regression analyses. Twelve regression formulae constituting a combination of all 10 parameters were developed. The authors found that equation 10 showed the most promising results with the multiple correlation coefficients equal to 0.903 and the standard error of estimate equal to 6.1 years. When the equation was back tested on the sample the mean difference between the actual age and the estimated age was found to be 5.1 years.

Watanabe *et al.* (1998) made use of the femur to derive formulae for estimating age at death. A sample of 98 Japanese individuals (72 male and 26 female) with ages ranging from 43 days to 88 years was used. The authors stained the sections with Villanueva's bone staining powder and with thionin dye. The following parameters were observed:

1. Area of perfect osteons and Haversian canals
2. Maximum and minimum diameter of perfect osteons and Haversian canals
3. Perimeter of perfect osteons and Haversian canals
4. Type II osteons
5. Osteon fragments
6. Area of triangle

It was found that the parameters of the osteons showed a higher correlation with age than the parameters of the Haversian canals. The data was subjected to linear regression analysis, multiple regression analysis and stepwise regression analysis.

The results yielded standard error of estimates ranging between 3.16 and 11.50 years, which, according to the authors, are a great deal better when compared to other literature. The authors believe this could be due to the staining method applied, which makes identification, and distinguishing between the various structures in the bone matrix, less problematic.

The most recent published method is that of Maat *et al.* (2006) which involves, once again, the use of the anterior midshaft of the femur. A sample of 162 Dutch/West European/Caucasian/White individuals was used of which 86 were male and 76 were female. The ages of the sample ranged from 15 years to 96 years and only individuals who were free from any chronic diseases were included.

Maat *et al.* (2006) made use of a Transmission light microscope with a 10x objective and 10x ocular lens. A framework of 10 x 10 squares on a transparent sheet was placed on an X-ray box, which was projected into the light microscope that was in turn reflected onto the bone slide. The framework allows a field size of exactly 1.0 x 1.0 mm^2 to be attained. A polarized lens was also used in the analysis of the slides and Maat *et al.* (2006) were specific about the zeroing of the polarized lens. The zeroing of the polarization filter enables the bone fibers of the unremodeled lamellar bone to stand out prominently, thus making it easier to distinguish between unremodeled bone and remodeled bone.

The data obtained from the analysis of the bone slides was subjected to regression analysis and six regression equations were formulated. The highest r-squared value is 0.799 with the accompanying SEE value of ± 10.6 years. The lowest SEE value (± 9.162 years) obtained was when the cadaver length was taken into consideration. The regression equation does, however, comprise the lowest r-squared value.

Chapter 3: Materials and Methods

3.1. Skeletal sample

The sample used in this study comprised of sections taken from the femoral shafts of 146 black South Africans (105 male and 41 female). The sample was randomly selected from the Student Bone Collection which is housed in the Department of Anatomy at the University of Pretoria. The collection is comprised mainly of black, males with very few females and even fewer individuals under the age of 25. Therefore an equal number of males and females or equal numbers from all age categories could not be selected. In addition, only the bones that had been previously numbered were used (i.e., whose identities were known). Bones showing signs of pathology were excluded from the study, as pathology may have had an effect on the results. Within these parameters, however, an attempt was made to obtain a sample which had both males and females, and covered all age ranges.

The skeletal collection at the University of Pretoria started in 1946 for the purpose of scientific research and training (Lewis G, pers comm). Cadavers were received from various donors and hospitals for medical dissection training. After the dissections were completed the material was macerated and placed into the bone collection for further research. Students have had access to skeletal material throughout the years.

The ages of the individuals used in this study are shown in Table 3.1. These ranged between 19 and 82 years with a mean age of 51.7 years. The age range for males was between 20 and 82 years (mean age = 52.3 years), while the females range was between 19 and 80 years (mean age = 50.4 years). As can be seen from the table, individuals from each decade were included. For the males, the sample included more than 10 individuals for each decade, except for the oldest (>80y) group. There was thus a good representation of all age categories. For the females, however, the number of individuals ranged from one to nine in the various age groups. Even though the sample size for females was small, it should be possible to make some general conclusions regarding possible microscopic differences between the sexes.

Although accurate age estimation techniques exist for juveniles and adolescents, one specimen younger than 20 years was included within the study to provide a clear indication of what the histological appearance of a young individual would be.

Table 3.1 Age distribution of study sample (SD = standard deviation, N = number of individuals)

AGE RANGE	TOTAL N	MEAN AGE	SD	MALE N	MEAN AGE	SD	FEMALE N	MEAN AGE	SD
< 20	1	19.0	-	-	-	-	1	19.0	-
20 - 29	15	24.2	2.8	12	24.5	2.9	3	23.0	1.7
30 - 39	21	35.1	2.8	15	34.7	2.7	6	36.3	2.7
40 - 49	27	44.7	3.2	18	45.2	3.1	9	43.8	3.3
50 - 59	28	53.4	2.9	20	53.9	3.0	8	52.3	2.7
60 - 69	26	63.8	2.7	18	64.2	2.5	8	62.8	3.2
70 - 79	23	72.7	3.2	19	72.9	3.2	4	71.3	2.5
80 - 89	5	80.4	0.9	3	80.7	1.2	2	80.0	0
TOTAL	**146**	**51.7**	**16**	**105**	**52.3**	**17**	**41**	**50.4**	**16**

3.2. Ethics

This research falls under the Human Tissue Act 65 of 1983, which specifies that an institution may use, for research purposes, tissues of an individual who has donated his/her body for this purpose. A proposal was submitted and the project was approved by the Students Ethical Committee.

3.3. Sampling procedure

Bone samples were removed from the anterior midshaft of each femur directly opposite the linea aspera. This method of bone removal falls along the anatomicall axis of the femur. The femur was chosen as it is very durable under extreme conditions, the most resistant to taphonomic influences and the cortical surface does not have muscle attachments, thus allowing a degree of biomechanical stability (Ericksen, 1991; Maat *et al*. 2006) Due to its durable nature, the femur is one of the most common bones found at crime scenes and archaeological sites. The femur (midshaft) has also been used in previous studies (e.g., Kerley, 1965; Ahlqvist and Damsten, 1969; Singh and Gunberg, 1970; Thompson, 1979; Ericksen, 1991; Maat *et al.* 2006).

The area of the midshaft from which the section was taken was measured and marked with a pencil. The femur was placed in a custom made jig (an apparatus to hold and stabilize the bone) and secured in order to reduce any additional movement (Figure 3.1). A small slab of bone (0.2 cm x 1.0 cm) was removed from the anterior midshaft of each femur, using a Ryobi router fitted with a 1.5 mm bit.

If any visible cracks were seen on the section that was to be taken, superglue was spread over the area to stabilize the bone, and then the sample was taken, according to the procedure described by Maat *et al.* (2003). Destruction of the bone is of great concern and thus the smallest possible sample was taken. This permits further use of the bones for demonstration purposes.

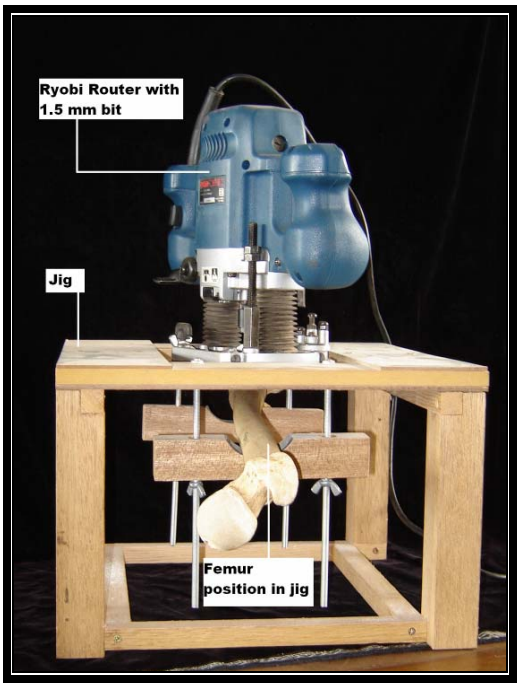


Figure 3.1 Illustration of the jig used to remove the bone samples

3.4. Section preparation

Bone slides were prepared using the method proposed by Maat *et al.* (2003).

Step 1: A sheet of waterproof abrasive paper (grit no. 220) was cut into half sections. A glass slab (13.8 x 22.8 cm) was greased with Vaseline and one half of the abrasive paper was stuck down (abrasive side up). The waterproof sheet was cut in such a manner that it overlapped the edges of the glass slab in order to prevent water from reaching underneath.

Step 2: The section of bone required for the study was removed.

Step 3: A central area of the abrasive paper was moistened with tap water. If the slice was greasy, a few drops of kitchen detergent was added. Once ready the section was ground by hand with a rotating motion until both sides were smooth and flat. Moderate pressure was applied during grinding and care was taken not let the slice topple.

Step 4: A section holder known as the "Frost's gripping device", was put together by folding a slip of fresh abrasive paper, with its abrasive side outward, transversely across the central part of the one side of a glass microscope slide. The two free ends of the slip were used to hold the device. This was done by placing the index finger in between the free ends on the centre of the glass slide and by positioning the thumb and middle finger of the same hand on the outward side of the same ends (Figure 3.2). The hand ground section was placed beneath the device and ground further (circular motion).

Figure 3.2 Illustration of Frost's gripping device

Step 5: Both sides of the section were ground down alternately by applying light to medium pressure with the "Frost's device" during the rotating motion. Grinding was started in the central area of the sheet where the abrasive paper was less rough and was gradually worked out to the rougher periphery.

The edges of the sheet were avoided as they could have been contaminated with Vaseline, which could have resulted in the edges turning up, and thus damaging the section.

Step 6: The section was ground down to its final thickness (± 50 - 100 microns) in the central area of the abrasive sheet. The bone section was ready when it had an opaque appearance and was transparent to the point that it was barely visible. The use of an extra abrasive sheet with finer grit was not necessary in this study.

Step 7: The final section was kept moist to prevent it from curling at the sides. The section was cleaned by immersing it in distilled water with a few drops of detergent. A small paintbrush was used to turn the section over underwater to make sure that it was cleaned on both sides. Cleaning underwater kept the section in a state of suspension and avoided any abrupt movements, which could have damaged the section. The distilled water was refreshed and the cleaning process was repeated three times.

Step 8: The cleaned section was lifted out of the water with a soft brush and placed to dry on a piece of filter paper in a Petri dish. Rumpled filter paper was used to facilitate the lifting of the section once it had dried completely.

Step 9: If necessary, the regular unembedded dry section could be used for any histological staining by placing it into a porcelain filtering cup and using it as a carrier.

Step 10: A glass microscope slide (76 mm x 26 mm) was cleaned with 90% alcohol and placed on a glass slab with some dark (preferably black) paper underneath. A permanent marker was used to number each slide according to the recorded number of the femur. A few drops of mounting medium were applied to the centre of the slide and the dried section was lifted using a pair of blunt-nosed tweezers and positioned on top of the mounting medium as soon as possible. More mounting medium was immediately added on top of the section to make sure that it was completely covered. If the section happened to move off centre, it was repositioned by pushing it with a needle. Without waiting, a glass cover slip was dipped

completely in Xylene and the excess Xylene removed by tapping the sides of the cover slip on some filter paper. The cover slip was then gradually lowered over the immersed section using a pair of pointed tweezers. The mounting medium automatically spreads itself beneath the cover slip. The slide was left to lie horizontally for one or two days before it was stored in a slide box.

3.5. Microscopic analysis of bone slides

The bone slides were examined under a transmission light microscope (Nikon) with a 10x objective and 10x ocular lens. The Nikon microscope was also fitted with a camera and a polarizing plate. The field length obtained by the combination of the microscope and the magnification of the camera was 0.795 mm x 0.636 mm (field size of photograph). The bone slides were marked with a permanent marker at five measured points just above the periosteal edge of the bone. The points were marked at 0 mm, 2.5 mm, 5 mm, 7.5 mm and 10 mm (Figure 3.3). This enabled five constant fields to be selected on each sample on the outer third of the periosteal edge. Digital photographs of each of the five fields were taken for the observations. If uncertainty occurred, the marked points allowed the observer to re-examine the same location on the slide. The program, ACT-1 for L-1 Version, was used to take the digital photos. The final area per field was measured in Image Tool (Version 3.00, Copyright © 1992-2002 UTHSCSA.) and was found to be 0.5062 mm^2, thus making the combined fields of observation, when multiplying by five, 2.53 mm^2.

The advantages of using photographic analysis of the bone sections are ably described in Ericksen's (1991) article and include:

1. The field is arbitrarily defined. The slide can be shifted to observe structures on the periphery without loosing the field.
2. The same field can repeatedly be found and the photos can be kept as a permanent record
3. Structures can be outlined and labelled on the photos.
4. Photos can supplement direct vision.

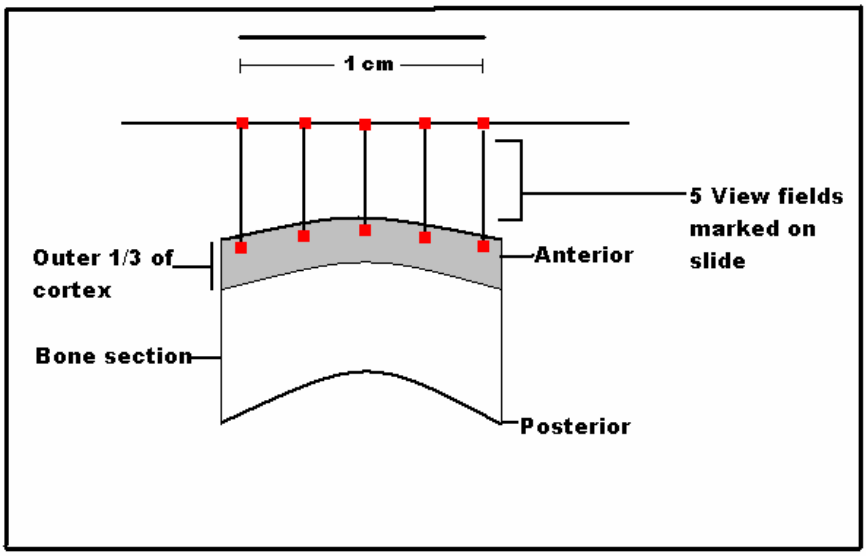


Figure 3.3 Illustration of the 5 marked points of observation on the bone slide

Ten observations were made and included criteria previously published by various authors (Kerley 1965, Singh and Gunberg 1970, Ericksen 1991). Numerous observations were selected in order to determine which characteristics demonstrated the highest correlation with age. These observations have been summarized in Table 3.2.

Table 3.2 Brief summary of the variables, including the abbreviations

Number	Variable	Abbreviation
1	Total number of osteons (for measurement purposes)	**Tot_osteons**
2	Total number of osteons (for non-measurement purposes)	**Tot_ost_mea**
3	The average number of lamellae counted per osteon	**Lam_ost**
4	The average Haversian canal diameter per osteon	**Hav_can**
5	The number of non-haversian canals	**Non-Hav_can**
6	The total number of osteon fragments	**Ost_frag**
7	The total number of resorption spaces	**Res_spac**
8	The average percent of osteonal bone	**%Ost_bone**
9	The average percent of unremodeled bone	**%Unr_bone**
10	The average percent of fragmental bone	**%Frag_bone**

These observations were:

1. **Total number of osteons (non-measurement purposes):** This count was done so as not to exclude any osteons in the field where the measurement (canal diameter and lamellae count) could not be taken. This count included all positively identified osteons whether the canal was obscured or clearly visible (Figure 3.5). The osteons on the periphery of the picture were only included if they could be identified as being either a secondary or Type II osteon. This was done to make sure that an accurate count of all the osteons present in the view fields was made.

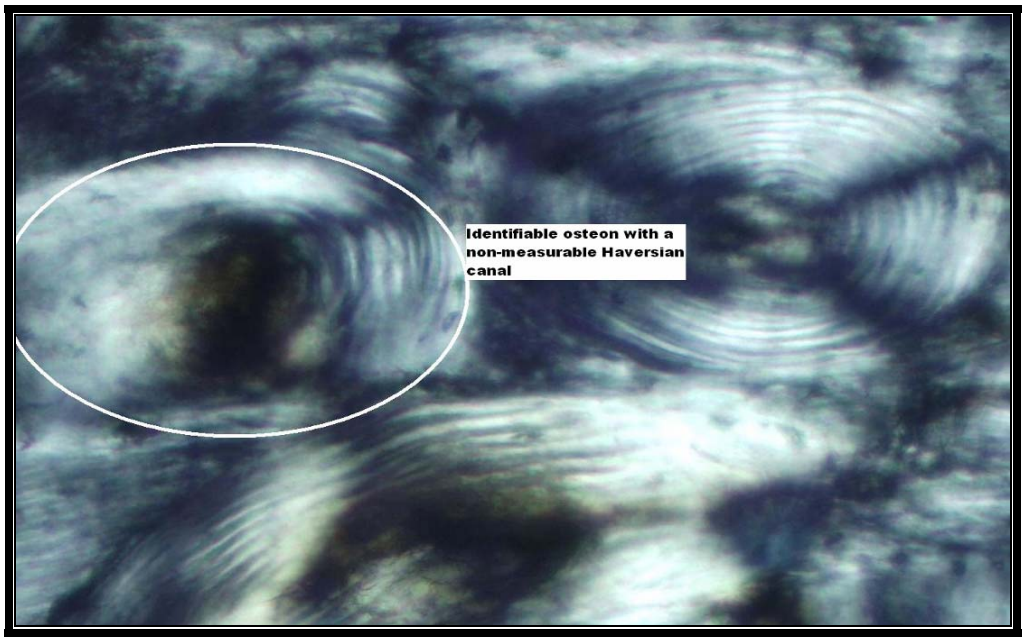


Figure 3.4 Diagram illustrating an osteon that is identified with a Haversian canal that is non-measurable

2. **Total number of osteons (for measurement purposes):** This count included all secondary and type II osteons only if it was possible to measure the minimum Haversian canal diameter of the osteons (Figure 3.4.). The osteons or semi-osteons seen at the periphery of the photograph were not counted (unless a minimum canal diameter could be measured), but were instead included in a different count when the percentage of osteonal bone count was

determined. If the Haversian canal was completely obscured by a Volkmann's canal that osteon was also excluded from the count. If the Volkmann's canal did not obscure the form of the Haversian canal to the extent where the two canals were indistinguishable, then that osteon was counted and measured. This count included all these osteons in all of the fields.

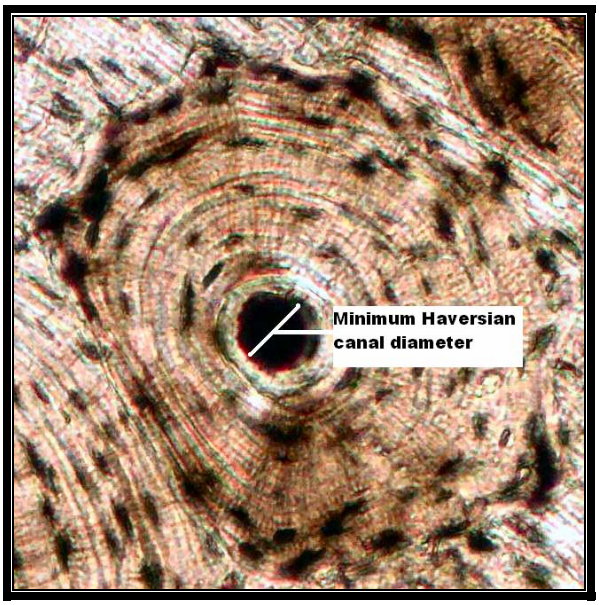


Figure 3.5 Haversian system displaying a measurable Haversian canal

3. **Average number of lamellae per osteon:** The total number of lamellae were counted in each recorded osteon, in all fields, and then averaged. Adding all the lamellae of all the counted osteons together and then dividing by the total number of fields, which are five, calculated the average number of lamellae per osteon. This count was done strictly by using the polarized light pictures so as to prevent any confusion in the case of cortical drift or osteon fragments (Figure 3.6.). The maximum numbers of lamellae were counted, starting from the innermost dark ring to the last dark ring at the first solid edge of the osteon. In cases where the lamellae were unclear, the plain light microscope picture was used in conjunction with the polarized light picture as a means of comparison.

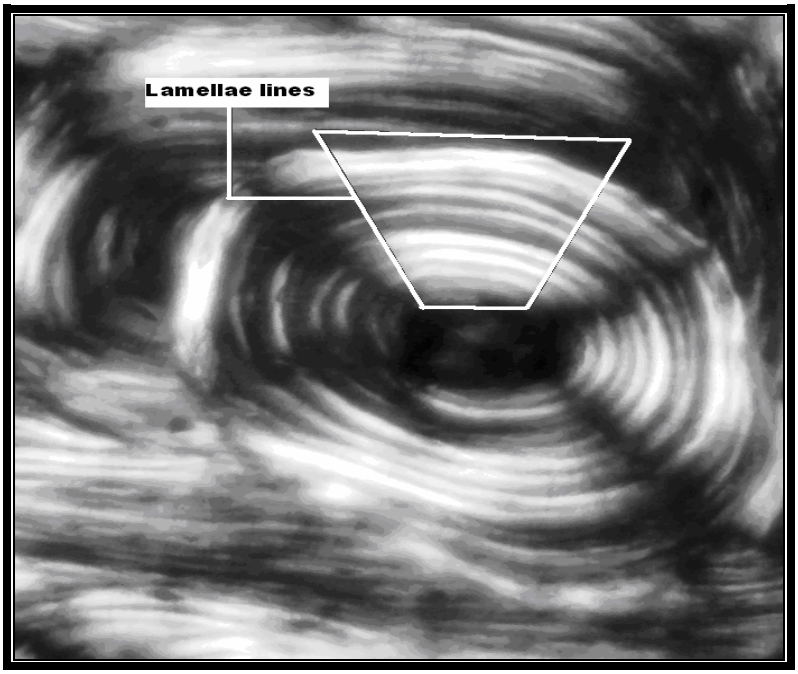


Figure 3.6 Lamellae lines counted with the use of a polarized filter

4. **Average Haversian canal diameter (µm):** All minimum osteon canal diameters (Figure 3.4.) were measured, in microns, by using Image Tool (Version 3.00, Copyright © 1992-2002 UTHSCSA.). Each canal diameter from all 5 fields was measured and added together to obtain the total sum of the Haversian canal diameters. The average was calculated by dividing the total sum of the canal diameters by the total number of measured osteons.

5. **The number of non-haversian canals:** These structures included all non-haversian canals, even those which were partly filled with concentric lamellae, which are known as primary osteons (Figure 3.7.)

6. **Total number of osteon fragments:** Osteon fragments were identified as being remnants of old osteons (Figure 3.8). When bone is remodeled, osteons are formed and as age increases new osteons are remodeled over the old ones. When this happens, the new osteon may completely cover the old Haversian canal of the old osteon, and leaves behind a small piece of the original osteon, partially visible behind the newly formed osteon. Osteon fragments can be either primary or secondary (Figure 3.8). Primary osteon fragments are

remnants of the first osteon that was laid down in the lamellar bone. The secondary osteon fragments are the remains of an osteon that was laid down over the first osteon and has now been remodeled by a new osteon. The osteons seen on the periphery of the photo were not included but were instead left for later counts (adapted from Ericksen, 1991). Care was taken not to confuse a drifting osteon with an osteon fragment (Figure 3.9).

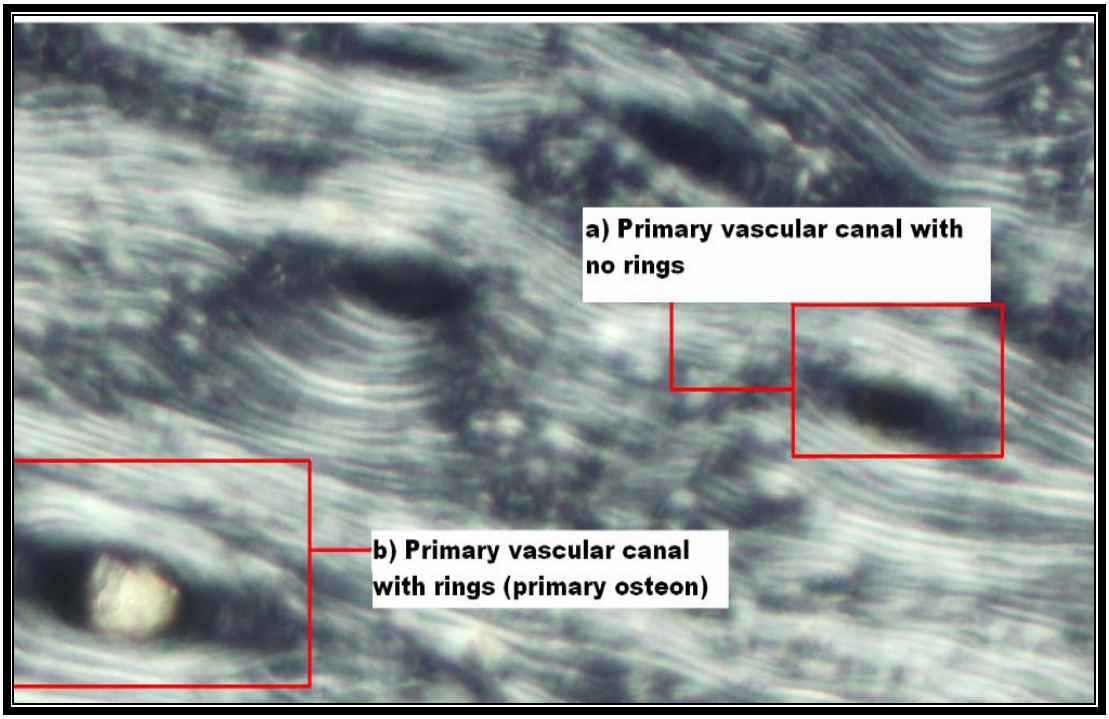


Figure 3.7 Illustration of a) primary vascular canal without concentric rings and, b) primary vascular canal with rings (primary osteon)

7. **Resorption spaces:** These include the first signs of new osteon formation and are characterized by scalloped edges. Resorption spaces can be either large or small depending on their stage of resorption (Figure 3.10.). Small resorption spaces must not be confused with non-haversian canals.

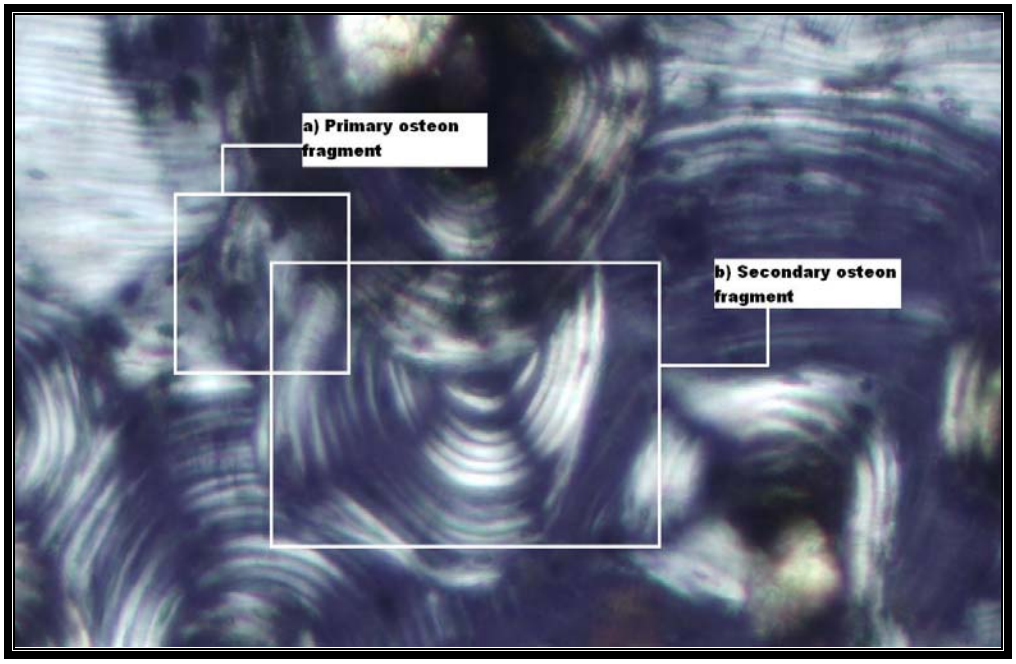


Figure 3.8 Illustration of a) primary osteon fragment and a b) secondary osteon fragment

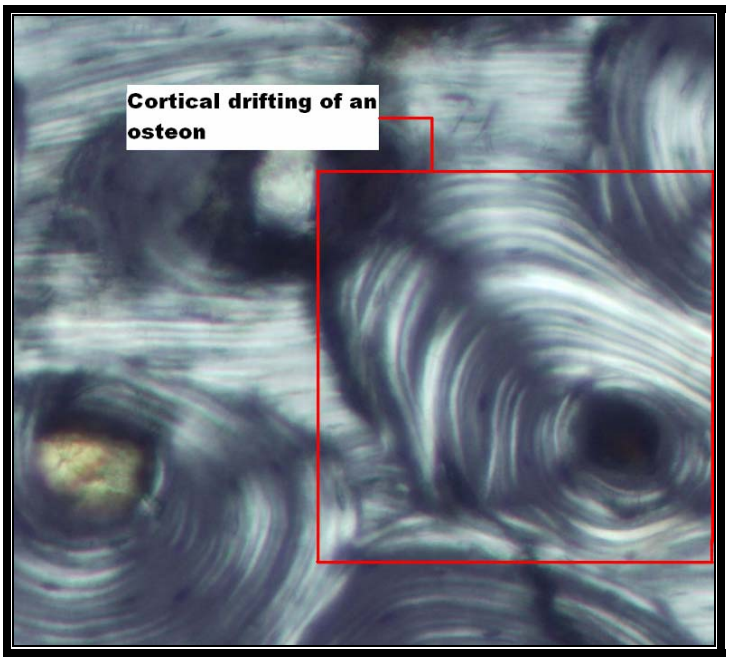


Figure 3.9 Illustration of cortical drifting

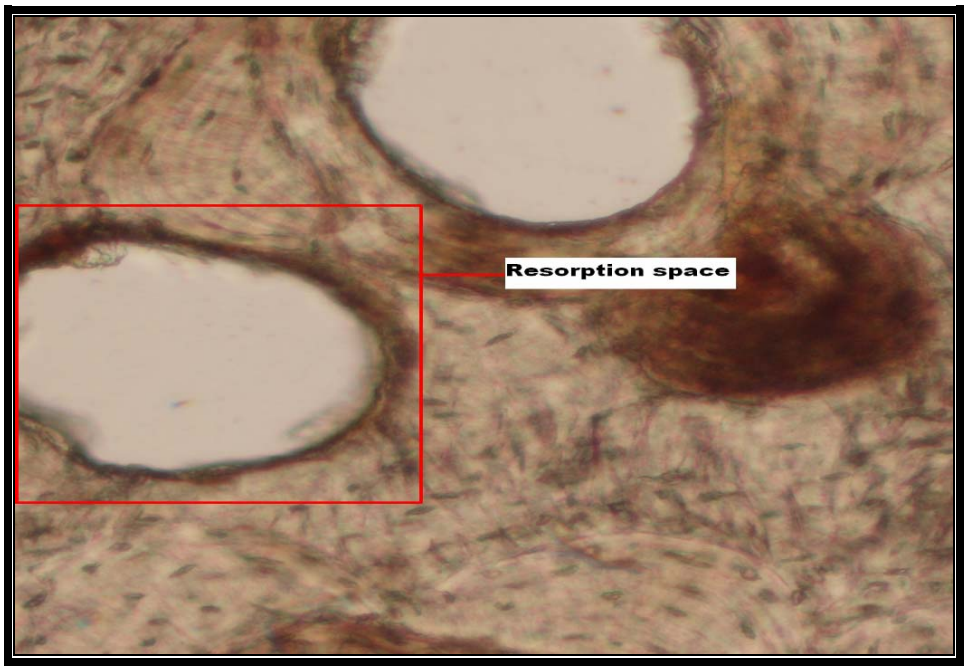


Figure 3.10 Illustration of a large resorption space

The following three observations were made by placing a 100-space, square grid over the photo. Each block was counted and allocated one of the three types of bone depending on which structure was predominant in that block. The three types of bone are explained below. The counts were then added together and divided by the total number of fields, which is 5, to get the average percentage.

8. **Percentage of osteonal bone (%):** This refers to all remodeled bone and includes osteons that were previously seen on the periphery of the photo (Ericksen, 1991).

9. **Percentage of unremodeled lamellar bone (%):** Unremodeled lamellar bone is the bone that has not yet undergone the process of remodeling by the formation of Haversian systems (Ericksen, 1991). This also included all non-haversian canals.

10. **Percentage of fragmental bone (%):** This included all osteon fragments visible within the fields of observation (Ericksen, 1991).

3.6 Statistical analysis

Insight into the relationship between two variables is best achieved by using correlation and regression analysis. Correlation analysis indicates the strength between two variables and regression analysis provides the mathematical equation used to predict one variable having the known value of the other variable (Greenfield *et al.*, 1998). When the data has been recorded a scatterplot graph is usually created, as a means to visually illustrate the relationship. At a univariate level the dependent factor, in this case, age, is plotted on the y-axis and the varying factor is plotted on the x-axis. This indicates that an increase or decrease in a given parameter (x-axis) either results in an increase or decrease in age which is plotted on the y-axis (Becker P, pers comm).

A correlation coefficient (r) is established to summarize the strength of the linear relationship between the variables (Greenfield *et a.*, 1998). According to Allan (1982), a correlation between 0.75 – 0.99 is considered high, a correlation between 0.5 – 0.74 is considered moderate and a correlation between 0.25 – 0.49 is low (Allan, 1982). There are a few aspects of correlation coefficients that must be taken into account before the results are interpreted. The correlation coefficient does not, on its own, explain the causal relationship between two variables. A causal relationship just means that the value of one variable will cause or produce a change in the other variable (Allan, 1982). It should also be noted that the significance of the r value is highly dependent on sample size. The best way to interpret correlation coefficient is to calculate the coefficient of determination (r^2). This value determines the proportion of variation in y-values which is accounted for by variation in the x-values (Allan, 1982).

The closer the correlation coefficient is to 1.0, the stronger the positive relationship is between the variables, and the closer the correlation coefficient is to -1.0 the stronger the negative relationship is. Once a linear relationship has been established, it is important to determine whether that relationship is statistically significant or not (Greenfield *et al.*, 1998). This is accomplished by determining the coefficient of determination (r^2) (Becker P, pers com). The coefficient determines the proportion of variation in the dependent variable that can be explained by the variation in the independent variable (Greenfield *et al.*, 1998).

Regression analysis provides information about the change in magnitude between the variables (Greenfield *et al.*, 1998). There are different types of regression analyses but in this study only three will be used, simple, curvilinear and multiple linear regression analysis. Simple linear regression enables the researcher to observe the relationship of one predictor variable with the outcome variable whereas multiple linear regression analysis is characterized by observing the simultaneous effect of multiple variables on one outcome variable (Greenfield *et al.*, 1998). For the linear regression analysis the formulae are presented in the following form: *[y = mx + c]*

Age = (slope x variable) + intercept ± SEE (standard error of the estimate)

In this study, the predictor (femur) variables, that at a univariate level are significantly correlated with the outcome variable at the 0.20 level of significance, will be included in the modeling process of age onto the femur parameters (Becker P, pers comm). The resulting multiple regression equations will then be assed in a cross-variation with testing done at the 0.05 level of significance (Becker P, pers comm).

The standard error of the estimate (SEE) is also determined to allow calculation of a standard error range for each of the regression formulae. The importance of determining the SEE is that it allows the recorder to draw a line parallel to the regression line which indicates the values of *y* that lie within the probabilities of 68%, 95% and 99% of the regression line (Allan, 1982). This allows or the predication of the *y*-value and the distance in which it lies from the regression line (Allan, 1982). The equation (Tedeschi, 2006) used to determine SEE is as follows: ^{n}

$$\mathrm{MSE} = \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{n-2}$$

MSE: Mean square error OR standard error of the estimate

n: Sample size

Y_i: This is the *i*th observed or measured value

$\hat{Y}_i$: Predicted values by the statistical regression model

3.6.1 Intra- and Interobserver error (repeatability tests)

Repeatability tests were conducted. The aim of intra- and interobserver error tests is to test the repeatability of the proposed method. The intraobserver error was done by the primary observer and determines whether the observer is able to repeat his/her own results. The interobserver error was done by an external observer and tests whether the results can be reproduced by anyone trying to make use of the proposed method. If the correlation of a proposed age estimation technique is high, but the observer error yields a low correlation, then the method may not be practical in accurately estimating age.

In order to test the repeatability, 16 slides were randomly chosen (male and female) and re-analyzed and compared to the original results. The time between re-analysis varied from a month to six months. The interobserver scoring was done by an experienced histologist.

Chapter 4: Results

4.1 Linear regression analysis (Univariate analysis)

In the first section of the results for this study each independent variable will be discussed and simple scatter plot graphs for each of these variables are presented in Figures 4.1 to 4.10. The scatter plot graphs give a visual indication of what the correlation of these variables are with age. One graph for each of the variables is given and this includes the data for both the males and females (sex-pooled). Separate graphs for the male and female samples were not included as the difference between these two groups was negligible.

4.1.1 Relationship between age and the total number of osteons counted (non-measurable)

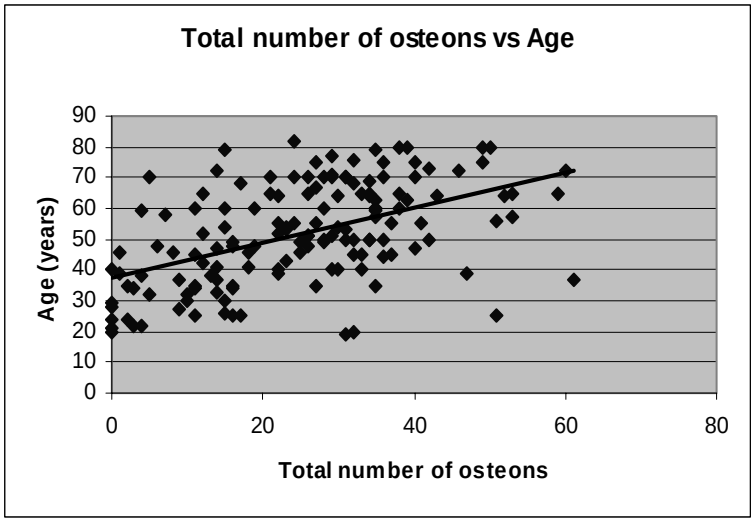


Figure 4.1 Scatterplot representing the correlation between the total number of osteons (non-measurable) and age (sexes pooled)

Figure 4.1 indicates that there is a positive correlation (*r*) between the total number of osteons (non-measurable) and age, when the sexes are combined. The r-value for this variable is 0.5041, indicating a moderate correlation with age (Table 4.2). Although there seems to be good positive correlation graphically, the r-squared value (coefficient of determination) is low. The coefficient of determination is 0.2541

which means that only about 25% of the variation in age can be explained by the total number of osteons present (Table 4.3). Statistically the coefficient of determination indicates the proportion of variation in the *y*-values which is accounted for by the variation in the *x*-values (Allan, 1982). The standard error of the estimate for this regression is ± 14.16 years, which is a very wide range for estimating age at death.

4.1.2 Relationship between age and the total number of osteons (measurable)

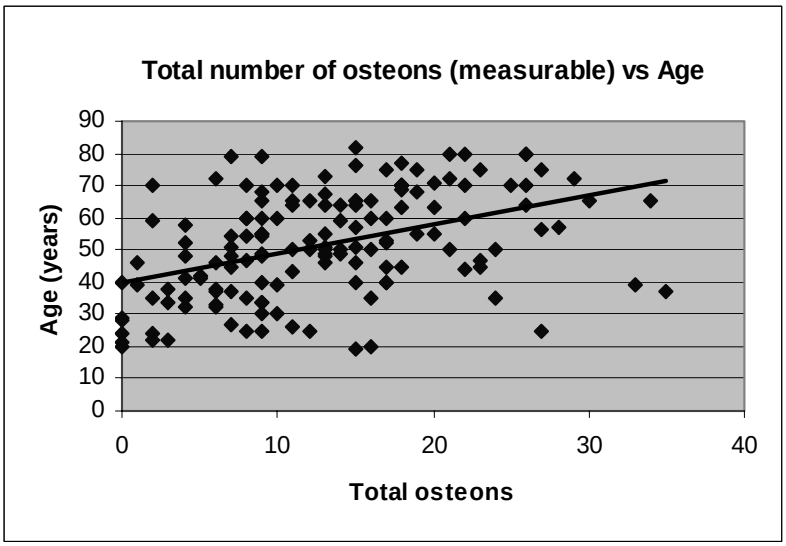


Figure 4.2 Scatterplot representing the correlation between the total number of osteons (measurable) and age (sexes pooled)

The results of the total number of measurable osteons (Figure 4.2) indicate that there is an increase in these structures, which is to be expected seeing that the non-measurable osteons also increased with age (Figure 4.1). It can be noted that the results are slightly better when all of the osteons in the field are counted indicating that even though the osteons are not measurable, it is better to count all of them to achieve a higher correlation. The correlation achieved for this variable (Tot_ost_mea) is r = 0.4274, which is an approximately 8% lower correlation than when all the osteons are counted. The coefficient of determination (r^2) is 0.1827 and the SEE is ± 14.82 years (Table 4.3).

4.1.3 Relationship between age and the average number of lamellae per osteon

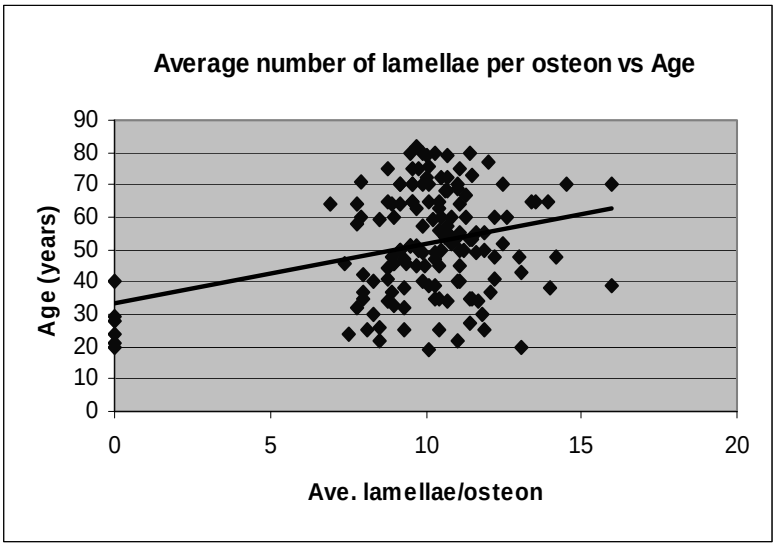


Figure 4.3 Scatterplot representing the correlation between the average number of lamellae per osteon and age (sexes pooled)

In Figure 4.3 it can be seen that there is a positive correlation (r = 0.2940) of the average number of lamellae per osteon with age, but if the r-squared value is taken into account, this variable proves to be a poor determining factor of age. The r-squared value and the SEE value obtained are r = 0.0864 and SEE ± 15.67 years respectively (Table 4.3). The cluster of points seen in the graphical representation of this variable against age is a clear indication that the number of lamellae per osteon is probably not age dependent. If the results are examined carefully it indicates that the average number of lamellae per osteon is approximately between 7 – 14 lamellae, regardless of the age.

4.1.4 Relationship between age and the average, minimum Haversian canal diameter per osteon

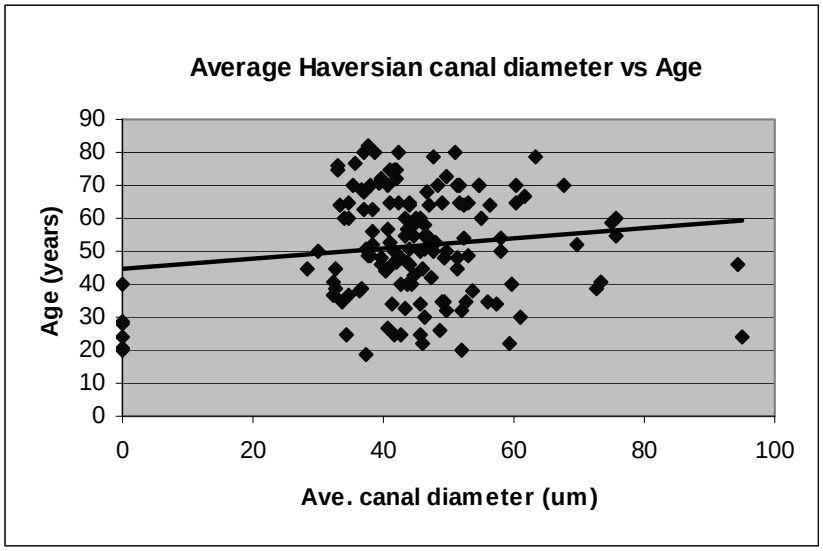


Figure 4.4 Scatterplot representing the correlation between the average Haversian canal diameter per osteon and age (sexes pooled)

The results of the correlation for the average Haversian canal diameter indicates that there is a slight positive correlation (r = 0.1377) with age (Figure 4.4), but the r–squared value is low, similarly to what was seen with the average number of lamellae per osteon. The r–squared value is 0.0109 and the SEE value is ± 16.24 years (Table 4.3). If the coefficient of determination is taken into account, it is clear that this variable is ineffective as a determinant of age. As seen with the average number of lamellae, it seems that there is a certain range of diameter size encountered despite the age of the individual. The average, minimum Haversian canal diameter ranges between 30 and 70 microns.

4.1.5 Relationship between age and the total number of non-haversian canals

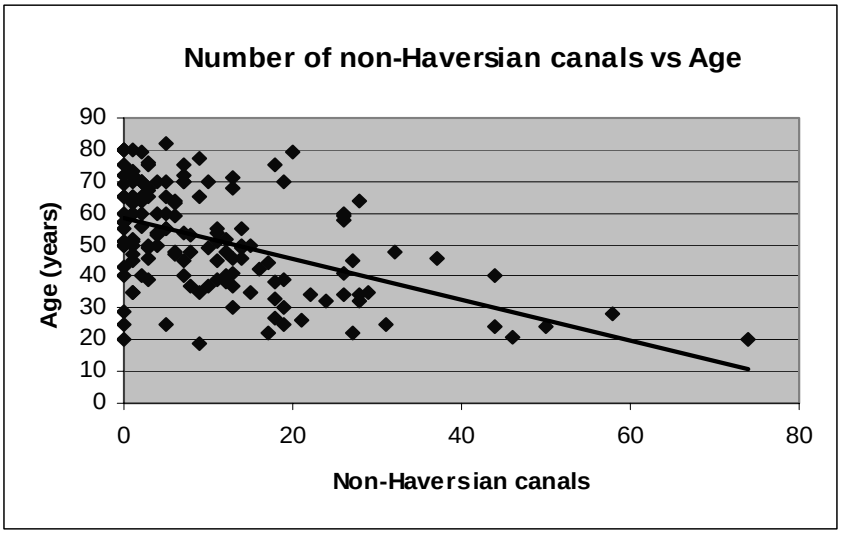


Figure 4.5 Scatterplot representing the correlation between the total number of non-haversian canals and age (sexes pooled)

The number of non-haversian canals, when plotted against age, displays a steep negative gradient thus indicating that the total numbers of these structures decrease with age (Figure 4.5). Although there is a steep decrease, the coefficient of determination (r^2) is relatively low. The r-squared value is 0.2447 and the SEE is ± 14.25 years (Table 4.3). When the male and female sample data was analyzed separately, it was found that the highest number of non-haversian canals for the males was 74 (age = 20 years) and the highest number in the females was 27 (age = 22 years). This is interesting to note as it is not clear or known why the female individuals showed a much lower number of non-haversian canals than the male sample at roughly the same age.

4.1.6 Relationship between age and the total number of osteon fragments

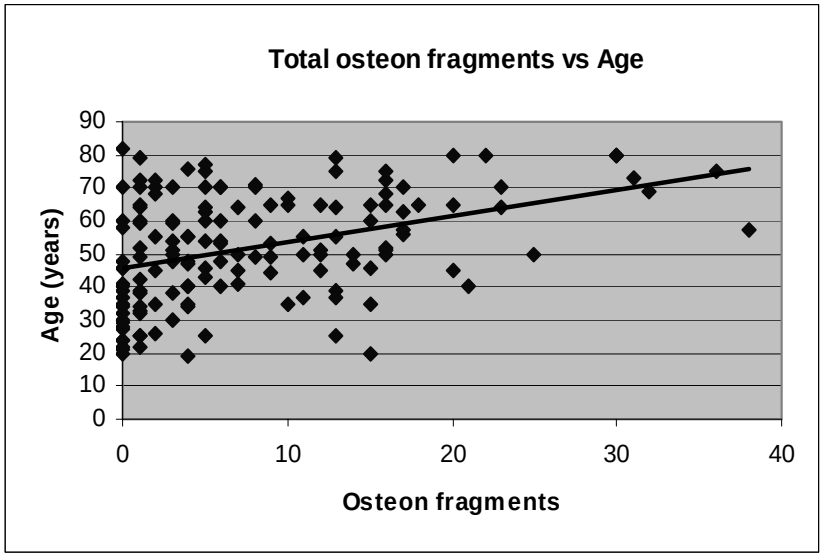


Figure 4.6 Scatterplot representing the correlation between the total number of osteon fragments and age (sexes pooled)

From the scatterplot of the total number of osteon fragments (Figure 4.6), it can be seen that the total number of these fragments tend to increase with age. The graph indicates a clear positive slope (r = 0.3977) but once again the r-squared value for the entire sample is low (0.1582) with a SEE value of ± 15.04 years (Table 4.3). It can be seen from the graph that there are a number of individuals of all ages in which none or only a few osteon fragments are observed.

4.1.7 Relationship between age and the total number of resorption spaces

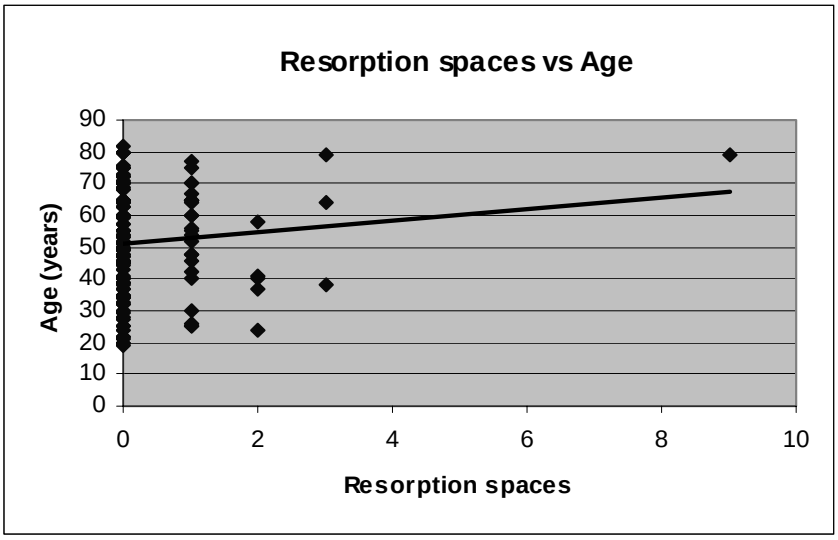


Figure 4.7 Scatterplot representing the correlation between the total number of resorption spaces and age (sexes pooled)

On the scatterplot (Figure. 4.7) it can be observed that the total number of resorption spaces ranged from 0 to 3, but with one individual having 9. The incidence of resorption spaces in this sample is extremely low with 77.4% of the sample having no resorption spaces at all. Out of the entire sample only one individual, aged 79 years, had a total of nine resorption spaces. When the male and female samples are viewed separately, it appears that females have a lower number of resorption spaces than males. The highest number of resorption spaces in the female sample is one whereas in the males it is nine. The coefficient of determination for this variable is 0.0119, indicating an extremely poor correlation with a standard error of the estimate of ± 16.30 years (Table 4.3). These two values confirm that this variable has no significant relationship with age and can not be used as a determinant for age at death estimation.

4.1.8 Relationship between age and the average percentage of osteonal bone

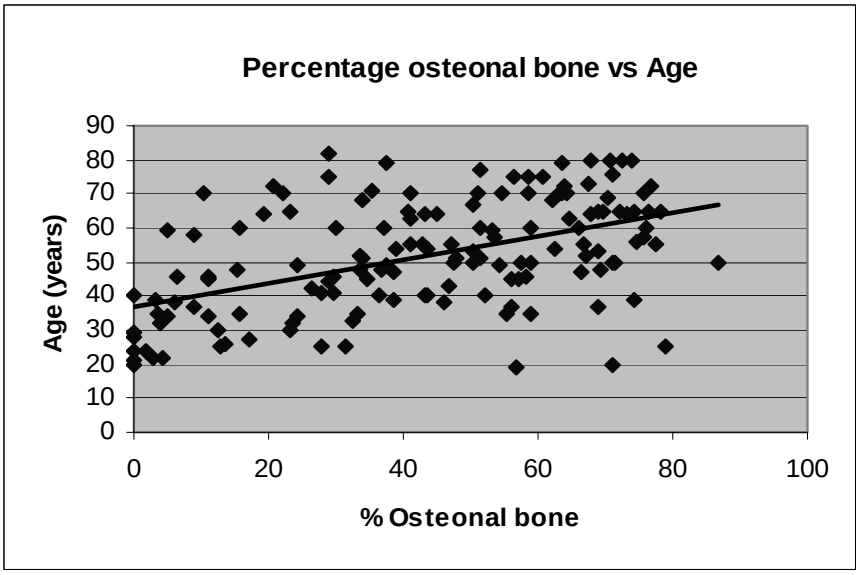


Figure 4.8 Scatterplot representing the correlation between the average percentage of osteonal bone and age (sexes pooled)

When the scatterplot is examined, it can be seen that the average percentage of osteonal bone increased with age (Figure 4.8). The r-value for this variable is 0.4941, indicating a moderate correlation. This is expected as the total number of osteons generally shows an increase with age. When the coefficient of determination (r^2 = 0.2441) is taken into account, it is seen that only 24% of the variation in age is explained by the percentage of osteonal bone (Table 4.3). The standard error of the estimate for this linear analysis is ± 14.26 years (Table 4.3).

4.1.9 Relationship between age and the average percentage of unremodeled bone

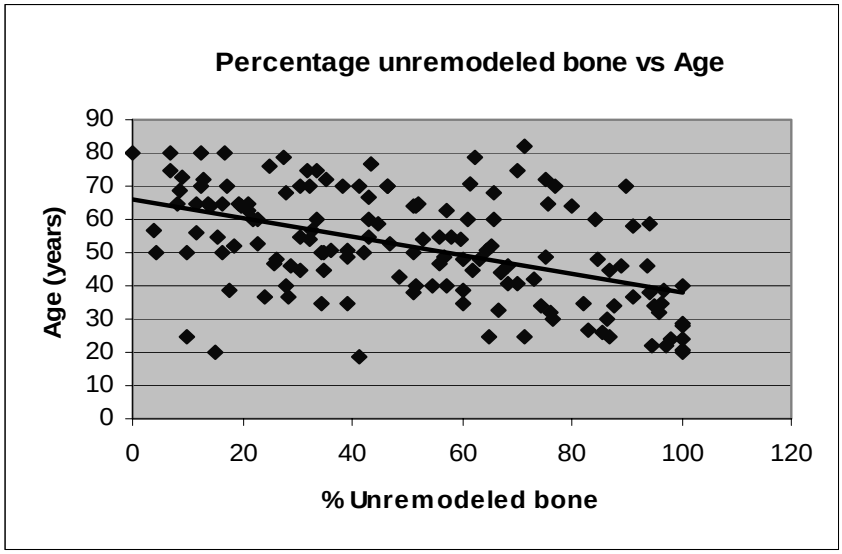


Figure 4.9 Scatterplot representing the correlation between the average percentage of unremodeled bone and age (sexes pooled)

When the scatterplot (Figure 4.9) is examined it is seen that the percentage of unremodeled bone decreases with age (r = -0.4984) which is to be expected. Once again the coefficient of determination is low (r^2 = 0.2484) and the standard error of the estimate (SEE) is high, being ± 14.22 years (Table 4.3).

4.1.10 Relationship between age and the average percentage of fragmental bone

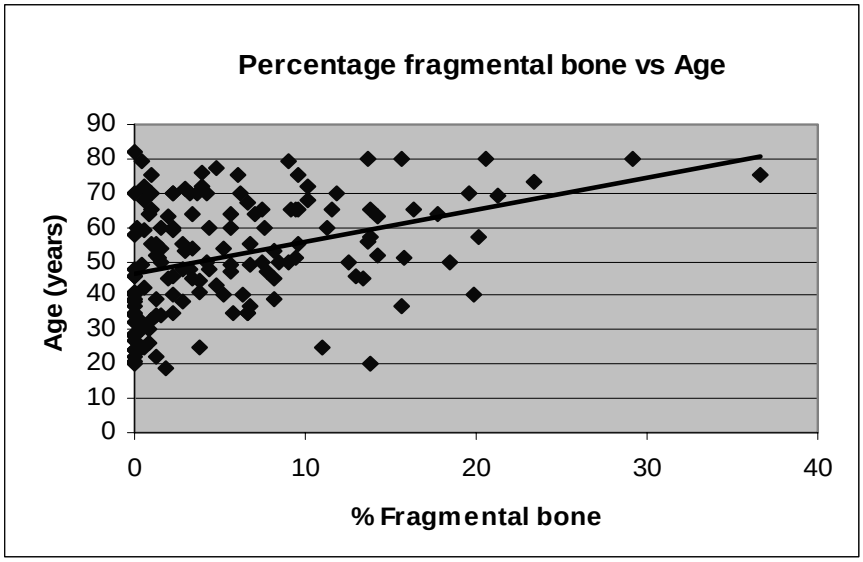


Figure 4.10 Scatterplot representing the correlation between the average percentage of fragmental bone and age (sexes pooled)

A strong positive correlation is seen when the percentage of fragmental bone is plotted against age (Figure 4.10) but the coefficient of determination is low. The r-squared value derived is 0.1381 and the SEE is ± 15.22 years (Table 4.3).

The above scatter plots are the graphical representation of the correlation between the individual variables and age. The male and female groups have been pooled together for the graphs as they do not present any significant difference between their correlations to display them separately (Table 4.1). Although the difference between the samples is small, it is recommended that sex-specific formulae be used if the sex is known.

The correlation coefficients for each of the individual variables are presented in Table 4.2. If the correlation coefficient is high it does not necessarily mean that the degree of correlation is high (Becker P. pers comm; Tedeschi, 2006). In this study the highest correlation coefficient is 0.547 (Table 4.2) and is seen for the percentage of osteonal bone. This is a moderate degree of correlation, indicating that there is a 55% correlation with age, but if the coefficient of determination of that same variable is analyzed it is observed to be 0.2992. This indicates that only 30% of the variance in age is accounted for by the variance seen in the percentage of osteonal bone,. This is

why high correlation coefficients should be no cause of excitement until the coefficient of determination has been derived.

Table 4.1 Graph indicating the non-significant difference observed between the male and female sample groups

Variable	Sample mean		Standard deviation		p - value
	Male	Female	Male	Female	
Tot_ost	24.46	26.78	14.81	13.25	0.382
Lam_ost	9.74	10.53	2.88	1.66	0.101
Hav_can.	44.3	45.27	16.01	9.28	0.716
Ost_frag	7.25	9.76	7.68	9.43	0.099
Res_spac	0.42	0.20	1.09	0.40	0.206
Non-Hav_can	12.19	6.66	13.72	7.64	0.016
%Ost_bone	41.58	47.35	24.22	22.30	0.189
%Unr_bone	53.06	45.04	28.78	27.53	0.128
%Frag_bone	5.26	7.57	5.77	7.97	0.055
Tot_ost_mea	12.64	14.17	7.81	8.09	0.293

Table 4.2 Correlation coefficients (r) of each independent variable when plotted against age, arranged in descending order for sex-pooled

Correlation coefficient (r)			
VARIABLE	**Sex-pooled (r)**	**Male (r)**	**Female (r)**
Tot_ost	0.5041	0.5291	0.4519
%Unr_bone	-0.4984	-0.5343	-0.4397
Non-Hav_can	-0.4946	-0.5448	-0.4344
%Ost_bone	0.4941	0.5469	0.3737
Tot_ost_mea	0.4274	0.4289	0.4504
Ost_frag	0.3977	0.3898	0.4633
%Frag_bone	0.3716	0.3510	0.4731
Lam_ost	0.2940	0.3707	0.0185
Hav_can.	0.1377	0.1646	0.0339
Res_spac	0.1092	0.1238	0.0003

Although the male and female samples were pooled in the above scatterplots, the following linear regression tables represent the results of all three groups, namely, the sexes pooled (Table 4.3), the male sample (Table 4.4) and the female sample (Table 4.5) separately for single variables. This was done in order to observe the slight differences between the individual samples and when the samples are pooled. The formulae for the linear regression are represented in Table 4.3, 4.4 and 4.5 and include information pertaining to the variable, the intercept, the slope, the standard error, the t-value, the standard error of the estimate (SEE) and the r-squared value (coefficient of determination). The SEE is defined as being the unbiased estimator of the variance of the random error (Tedeschi, 2006) and is also known as the mean standard error (MSE). The raw data used to plot these graphs is given in Appendix A. The correlation coefficients for the linear equations derived from the graphs are provided in Table 4.2.

Table 4.3 Results of the linear regression analysis (single variable only) for pooled sexes (SEE = standard error of the estimate, r^2 = coefficient of determination, t value = students t-test)

Age = Intercept + [variable x slope] ± SEE						
Variable	**Intercept**	**Slope**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	37.36	0.5729	0.0818	7.005	14.16	0.2541
2. Tot_ost_mea	40.17	0.8855	0.1561	5.674	14.82	0.1827
3. Lam_ost	33.47	1.8355	0.4973	3.691	15.67	0.0864
4. Hav_can	44.79	0.1561	0.0935	1.668	16.24	0.0190
5. Non-Hav_can	58.59	-0.6441	0.0943	6.829	14.25	0.2447
6. Ost_frag	45.48	0.7874	0.1514	5.202	15.04	0.1582
7. Res_spac	51.08	1.8593	1.4108	1.318	16.30	0.0119
8. %Ost_bone	37.07	0.3397	0.0498	6.819	14.26	0.2441
9. %Unr_bone	66.23	-0.2851	0.0413	6.899	14.22	0.2484
10. %Frag_bone	46.24	0.9312	0.1939	4.803	15.22	0.1381

The above Table 4.3, shows the results when the male and female samples are pooled. The single variable that is best related to age for this pooled sexes sample is the total number of non-measurable osteons (Tot_ost). The coefficient of determination (*r2*) for this formula is 0.2541 and the standard error of the estimate is ± 14.16 years.

The following two tables (Table 4.4 and 4.5) display the results of the linear regression analysis for separate male and female sample groups. The male sample is best represented by the average percentage osteonal bone with a coefficient of determination of 0.2992 and the female sample group is best represented by the average percentage if fragmental bone (r^2 = 0.2238).

Table 4.4 Results of the linear regression analysis (single variable only) for the male sample (SEE: standard error of the estimate, r^2: coefficient of determination)

Age = intercept + [variable x slope] ±SEE						
Variable	**Intercept**	**Slope**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	37.75	0.5944	0.0939	6.328	14.19	0.2799
2. Tot_ost_mea	40.73	0.9141	0.1897	4.818	15.10	0.1839
3. Lam_ost	31.46	2.1390	0.5281	4.050	15.53	0.1374
4. Hav_can	44.71	0.1710	0.1010	1.694	16.49	0.0271
5. Non-Hav_can	60.34	-0.6604	0.1002	6.594	14.02	0.2968
6. Ost_frag	46.17	0.8444	0.1965	4.296	15.39	0.1520
7. Res_spac	51.50	1.8750	1.4804	1.267	16.59	0.0153
8. %Ost_bone	36.66	0.3757	0.0567	6.631	13.99	0.2992
9. %Unr_bone	68.67	-0.3089	0.0481	6.415	14.13	0.2855
10. %Frag_bone	46.96	1.0113	0.2658	3.805	15.65	0.1232

Table 4.5 Results of the linear regression analysis (single variable only) for the female sample (SEE: standard error of the estimate, r^2: coefficient of determination)

Age = intercept + [variable x slope] ±SEE						
Variable	**Intercept**	**Slope**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	36.05	0.5347	0.1690	3.164	14.16	0.2042
2. Tot_ost_mea	37.99	0.8729	0.2771	3.151	14.17	0.2029
3. Lam_ost	48.53	0.1743	1.5095	0.115	15.87	0.0003
4. Hav_can	47.77	0.0574	0.2704	0.212	15.86	0.0012
5. Non-Hav_can	56.29	-0.8907	0.2957	3.012	14.29	0.1887
6. Ost_frag	42.85	0.7702	0.2359	3.265	14.07	0.2147
7. Res_spac	50.36	0.0114	6.2551	0.002	15.87	0.0000
8. %Ost_bone	37.93	0.2626	0.1044	2.516	14.72	0.1397
9. %Unr_bone	61.64	-0.2503	0.0819	3.057	14.26	0.1933
10. %Frag_bone	43.33	0.9304	0.2774	3.353	13.98	0.2238

Maat *et al.* (2006) proposed that the remodeling of bone follows more of a curvilinear course than a linear one. Kerley (1965) also applied curvilinear analysis in his study to develop regression equations. The following tables (4.6 – 4.8) represent the results for curvilinear (quadratic) analysis applied to this sample. The formula that yielded the best results for the pooled sexes with regards to the coefficient of determination and the standard error of the estimate is the first formula, represented by the total number of non-measurable osteons (Tot_ost). The coefficient of determination was r^2 = 0.2970 and the SEE = 13.79. The formula with best coefficient of determination and standard error of the estimate for the male sample is also when the total number of non-measurable osteons (Tot_ost) is taken into account. This formula yielded the highest coefficient of determination (r^2 = 0.3766) and the lowest SEE = 13.26. The female sample yielded the best results when the formula was represented by the total number of osteon fragments (Ost_frag). The coefficient of determination was r^2 = 0.2422 and the SEE = 13.99.

Table 4.6 Results for the curvilinear analysis (single variable only) for pooled sexes (SEE = standard error of the estimate, r^2 = coefficient of determination, t value = students t-test)

Age (years) = Intercept + slope1 (variable) + slope2 (variable)^2							
Variable	**Intercept**	**Slope1**	**Slope2**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	30.873	1.2823	-0.0135	0.2510	5.11	13.79	0.2970
2. Tot_ost_mea	31.638	2.5047	-0.0543	0.4830	5.19	14.27	0.2481
3. Lam_ost	26.211	4.3883	-0.1714	1.3742	3.19	15.51	0.1110
4. Hav_can	29.030	0.9866	-0.0097	0.2414	4.09	15.57	0.1049
5. Non-Hav_can	59.626	-0.8548	0.0045	0.2186	-3.91	14.24	0.2506
6. Ost_frag	44.538	1.0938	-0.0114	0.3979	2.75	15.06	0.1622
7. Res_spac	51.568	-0.7322	0.4219	2.7982	-0.26	16.29	0.0198
8. %Ost_bone	31.821	0.7512	-0.0052	0.1865	4.03	14.05	0.2780
9. %Unr_bone	60.659	0.0128	-0.0028	0.1718	0.07	14.11	0.2648
10. %Frag_bone	45.393	1.2724	-0.0151	0.4557	2.79	15.24	0.1422

Table 4.7 Results for the curvilinear analysis (single variable only) for the male sample (SEE = standard error of the estimate, r^2 = coefficient of determination, t value = students t-test)

Age (years) = Intercept + slope1 (variable) + slope2 (variable)^2							
Variable	**Intercept**	**Slope1**	**Slope2**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	28.726	1.5996	-0.0191	0.2676	5.98	13.26	0.3766
2. Tot_ost_mea	31.183	2.7976	-0.0648	0.5337	5.24	14.23	0.2826
3. Lam_ost	25.472	4.7099	-0.1849	1.4771	3.19	15.34	0.1657
4. Hav_can	28.744	1.0609	-0.0106	0.2441	4.35	15.43	0.1564
5. Non-Hav_can	61.447	-0.8629	0.0041	0.2476	-3.49	14.03	0.3023
6. Ost_frag	44.017	1.5501	-0.0267	0.4770	3.25	15.27	0.1733
7. Res_spac	52.076	-0.9302	0.4372	3.0943	-0.30	16.58	0.0255
8. %Ost_bone	29.886	0.9431	-0.0073	0.2121	4.45	13.56	0.3482
9. %Unr_bone	58.011	0.2417	-0.0051	0.2008	1.20	13.68	0.3371
10. %Frag_bone	45.728	1.5975	-0.0305	0.6429	2.49	15.65	0.1317

Table 4.8 Results for the curvilinear analysis (single variable only) for the female sample (SEE = standard error of the estimate, r^2 = coefficient of determination, t value = students t-test)

Age (years) = Intercept + slope1 (variable) + slope2 (variable)2							
Variable	**Intercept**	**Slope1**	**Slope2**	**Std. error**	**t value**	**SEE**	**r^2**
1. Tot_ost	41.495	-0.0301	0.0109	0.6262	-0.05	14.18	0.2222
2. Tot_ost_mea	35.033	1.3823	-0.0161	1.1335	1.22	14.32	0.2074
3. Lam_ost	131.44	-14.734	0.6522	13.971	-1.05	15.84	0.0298
4. Hav_can	60.794	-0.4898	0.0055	2.0001	-0.24	16.06	0.0032
5. Non-Hav_can	57.275	-1.3775	0.0223	0.8835	-1.56	14.42	0.1960
6. Ost_frag	45.310	0.0009	0.0277	0.6956	0.00	13.99	0.2422
7. Res_spac	50.364	0.0114	-	6.2551	0.00	15.87	0.0000
8. %Ost_bone	38.129	0.2498	0.0001	0.3952	0.63	14.91	0.1397
9. %Unr_bone	67.681	-0.6036	0.0036	0.3173	-1.90	14.19	0.2205
10. %Frag_bone	43.146	0.9881	-0.0021	0.7095	1.39	14.17	0.2240

Table 4.9 demonstrates the differences observed when the data was subjected to curvilinear analysis and how the r-squared values compare to the linear regression. The correlations between the pooled sexes differ with a percentage between 0.4 – 8.59%, indicating that the curvilinear analysis improves the results by a slight degree. If the correlations between the male sample is compared, it is clear that the correlation for some of the variables increase between 10% (Total osteon count (measurable) and total osteon count (non-measurable)) and 13% (Haversian canal diameter). The remainder of the variables for the male sample increased between 0.55 – 9.87%. Results of the female sample improved with the curvilinear analysis between 0.00 – 2.75%, clearly demonstrating that the female group improved the least when compared to the pooled sexes and the male sample.

It is clear when the results of the curvilinear analysis are compared to that of the linear regression analysis, that the results improved, in some instances greatly, when curvilinear analysis was applied. The results indicate that the male sample differentially increases in accuracy when the curvilinear data were used. It is thus recommended that if the sex of the identified remains can be determined, that the sex-specific formula be applied for optimum results.

Table 4.9 Comparison of the coefficients of determination (r^2) between linear analysis and curvilinear analysis

	Sex-pooled		Male		Female	
Variable	**Linear**	**Curvilinear**	**Linear**	**Curvilinear**	**Linear**	**Curvilinear**
	r^2	**r^2**	**r^2**	**r^2**	**r^2**	**r^2**
1. Tot_ost	0.2541	0.2977	0.2799	0.3766	0.2042	0.2220
2. Tot_ost_mea	0.1827	0.1422	0.1839	0.2826	0.2029	0.2074
3. Lam_ost	0.0864	0.1110	0.1374	0.1657	0.0003	0.0298
4. Hav_can	0.0190	0.1049	0.0271	0.1564	0.0012	0.0032
5. Non-Hav_can	0.2447	0.2506	0.2968	0.3023	0.1887	0.1960
6. Ost_frag	0.1582	0.1622	0.1520	0.1733	0.2147	0.2422
7. Res_spac	0.0119	0.0198	0.0153	0.0255	0.0000	0.0000
8. %Ost_bone	0.2441	0.2708	0.2992	0.3482	0.1397	0.1397
9. %Unr_bone	0.2484	0.2648	0.2855	0.3371	0.1933	0.2205
10. %Frag_bone	0.1381	0.1422	0.1232	0.1317	0.2238	0.2240

4.2 Multiple regression analysis (multivariate)

Multiple regression formulae are presented in Tables 4.10 to 4.12 and contain the following information: the variable, the slope, the standard error, the intercept, the standard error of the estimate (SEE), and the r-squared value (coefficient of determination). Stepwise regression analysis and direct regression analysis was done in order to obtain formulae with the highest possible coefficient of determination (r^2) values. Stepwise regression analysis is when all the variables are entered into the relevant statistics program (eg., SPSS, v11.5) and the subsequent functions are done automatically by the program. Direct regression analysis involves the manual combining of various variables to achieve the highest possible coefficient of determination. Stepwise regression analysis was only used in Function A from the pooled sexes sample and the remainder of the formulae were derived via direct regression analysis. The regression formulae are presented in the following form, $[y = \sum (mx) + c]$, where,

$$\text{Age} = \text{Intercept} + \sum (\text{variable x slope}) \pm \text{SEE}$$

Table 4.10 represents the results of the multivariate analysis when the male and female samples were pooled. The functions are listed in the tables in descending order according to the coefficient of determination, in other words, from the highest correlated to the lowest correlated. Function A is the product of stepwise analysis and Functions B and C are the products of direct regression analysis. Function A is comprised of 6 of the 10 variables. In the multivariate analysis, the change in the coefficient of determination was minimal in comparison with the univariate analysis, with only a slight improvement in the SEE. The highest coefficient of determination was 0.3032 (Function A) whereas in the linear regression it was 0.2541 (sex-pooled). The best standard error of the estimate for the multivariate analysis was ± 13.84 years (Function C) whilst the linear regression analysis has a standard error of estimate of 14.16 years (Table 4.3). This indicates that there is a slight improvement in the standard error of the estimate and a 5% improvement when the coefficient of determination is taken into account. The question that remains is whether it is more practical to use the complex multiple regression formulae or the simpler, and less tedious linear regression formulae to estimate age at death.

Table 4.10 Results of the multiple regression analysis for pooled sexes (SEE: standard error of the estimate, r^2: coefficient of determination)

Age (years) = Intercept + ∑(variable x slope) ± SEE						
Function	**Variable**	**Intercept**	**Slope**	**Std. error**	**SEE**	**r^2**
A	**1. Tot_ost**	187.80	0.2994	0.1836	13.93	0.3032
	4. Ost_frag		0.0470	0.5094		
	6. Non-Hav_can		-0.3337	0.1437		
	7. %Ost_bone		-1.3993	1.3828		
	8. %Unr_bone		-1.4191	1.3815		
	9. %Frag_bone		-1.3279	1.4826		
B	**4. Ost_frag**	48.47	0.3031	0.1651	13.88	0.2980
	6. Non-Hav_can		-0.3375	0.1414		
	8. %Unr_bone		-0.0251	0.1172		
	9. %Frag_bone		0.0910	0.2976		
C	**1. Tot_ost**	46.75	0.3146	0.1292	13.84	0.2978
	4. Ost_frag		0.1124	0.1951		
	6. Non-Hav_can		-0.3571	0.1267		

The male multiple regression formulae show some degree of improvement when compared to the linear regression analysis. The best coefficient of determination achieved for the multivariate analysis is 0.4152 for Function A (Table 4.11). This is a 12% improvement on the linear regression formulae. This increase is only achieved when all the variables are used in the formulae. The improvement in the standard error of the estimate is only ± 0.68 years.

The results for the separate female multivariate analysis do not show much improvement when compared to the linear equations. The best coefficient of determination (r^2) obtained was 0.2860 (Table 4.12). This was achieved with the use of four of the ten variables. The highest r-squared value derived from the linear regression analysis was 0.2238 (Table 4.5). When this value (r^2 = 0.2238) is compared to the multivariate analysis for the female group it was observed that it was lower than all three of the values derived from the multiple regressions. This clearly indicates that the multiple regression yields improved results. The lowest standard error of the estimate derived from the multivariate analysis is ± 13.91 years which when compared to the lowest standard error of the estimate of the linear regression analysis (± 13.98 years) is not a significant change.

Table 4.11 Results of the multiple regression analysis for the male sample (SEE: standard error of the estimate, r^2: coefficient of determination)

Age (years) = Intercept + ∑(variable x slope) ± SEE						
Function	**Variable**	**Intercept**	**Slope**	**Std. error**	**SEE**	**r^2**
A	**1. Tot_ost**	155.73	0.1936	0.2132	13.31	0.4152
	2. Lam_ost		0.2598	0.7404		
	3. Hav_can		0.0219	0.1101		
	4. Ost_frag		0.4695	0.6031		
	5. Res_spac		3.5399	1.2479		
	6. Non-Hav_can		-0.3256	0.1682		
	7. %Ost_bone		-1.0060	1.3586		
	8. %Unr_bone		-1.1535	1.357		
	9. %Frag_bone		-1.8143	1.5804		
B	**1. Tot_ost**	176.63	0.1828	0.1886	13.70	0.3543
	6. Non-Hav_can		-0.3325	0.1553		
	7. %Ost_bone		-1.1317	1.3918		
	8. %Unr_bone		-1.3094	1.3905		
	9. %Frag_bone		-1.5628	1.4113		
C	**4. Ost_frag**	72.41	0.6432	0.5481	13.65	0.3532
	6. Non-Hav_can		-0.3644	0.1543		
	8. %Unr_bone		-0.2529	0.1109		
	9. %Frag_bone		-1.3148	0.7343		

Table 4.12 Results of the multiple regression analysis for the female sample (SEE: standard error of the estimate, r^2: coefficient of determination)

Age (years) = Intercept + ∑(variable x slope) ± SEE						
Function	**Variable**	**Intercept**	**Slope**	**Std. error**	**SEE**	**r^2**
A	**1. Tot_ost**	96.59	0.4135	0.3595	13.96	0.2860
	6. Non-Hav_can		-0.4540	0.4631		
	7. %Ost_bone		-0.6689	0.4147		
	8. %Unr_bone		-0.5019	0.3760		
B	**1. Tot_ost**	36.69	0.3833	0.2524	13.91	0.2711
	4. Ost_frag		-0.5144	0.9028		
	9. %Frag_bone		1.1145	0.9424		
C	**1. Tot_ost**	100.46	0.4019	0.3591	13.95	0.2670
	7. %Ost_bone		-0.6921	0.4138		
	8. %Unr_bone		-0.6236	0.3548		

4.3 Intra- and Interobserver error (repeatability tests)

One test that is capable of assessing the repeatability of a method is Pearson's correlation (correlation coefficient), which is presented in Table 4.13 (intraobserver) and Table 4.14 (interobserver). The ideal result for these tests is to obtain a value of 1.00, which would indicate a 100% repeatability of the method. An F-test was also conducted to assess whether there was any significant difference between the original results an the variables for the intraobserver and interobserver analysis. The raw data used to obtain these results can be viewed in Appendix B.

When the intraobserver results was analyzed (Table 4.13), the highest correlation seen in the sex-pooled sample was for the percentage of unremodeled bone (r = 0.9956) and the lowest was seen for the average lamellae per osteon (r = 0.7361). In the male sample the highest correlation was seen for the average Haversian canal diameter (r = 0.9964) and the lowest for the average lamellae per osteon (r = 0.7525). The female sample yielded the highest and the lowest correlation coefficients of 1.000 (resorption spaces) and 0.5599 (average lamellae per osteon) respectively. If the results of the F-test are examined, it was seen that the results are not significantly different. In general the most reliable variables to measure would be the average Haversian canal diameter, the percentage of unremodeled bone and percentage of fragmental bone; the most unreliable would be the average number of lamellae per osteon.

Table 4.13 Correlation coefficients and significant test (F-test) for the intraobserver results (SP = sexes pooled, M = male, F = female)

Variable	Correlation coefficient (SP)	F-test (SP)	Correlation coefficient (M)	F-test (M)	Correlation coefficient (F)	F-test (F)
Tot_ost	0.9777	0.8937	0.9810	0.9986	0.9599	0.8928
Tot_ost_mea	0.9723	0.5850	0.9761	0.6032	0.9639	0.7905
Lam_ost	0.7361	0.8914	0.7525	0.9719	0.5599	0.8063
Hav_can.	0.9928	0.7399	0.9964	0.7506	0.9867	0.7135
Non-Hav_can	0.9648	0.7849	0.9569	0.9097	0.9628	0.3399
Ost_frag	0.9721	0.6358	0.9292	0.8296	0.9934	0.6594
Res_spac	0.9524	0.9711	0.9453	0.9700	1.0000	1.0000
%Ost_bone	0.9909	0.8374	0.9948	0.9157	0.9681	0.7962
%Unr_bone	0.9956	0.7949	0.9951	0.9231	0.9951	0.7711
%Frag_bone	0.9745	0.3257	0.9304	0.8815	0.9912	0.4216

Table 4.14 shows the results of the interobserver error analysis. The external observer's results were compared to the original results and tabulated. When the external observer results were compared to the original results the highest correlation coefficient derived was 0.9815 (percentage unremodeled bone) and the lowest 0.1241 (average number of lamellae per osteon). Once again there is no significant difference observed in the intraobserver results.

In general the variable that proves to be the most repeatable was the percentage of unremodeled bone. The two lowest correlations were seen for the total number of resorption spaces and the average lamellae per osteon. The low repeatability for the resorption spaces can be attributed to the fact that there are limited resorption spaces observed throughout the sample, thus a miscount would lead to a dramatic influence on the results.

Table 4.14 Correlation coefficients and significance tests (F-test) for the interobserver results (SP = sex-pooled, M = male, F = female)

Variable	Correlation coefficient (SP)	F-test (SP)	Correlation coefficient (M)	F-test (M)	Correlation coefficient (F)	F-test (F)
Tot_ost	0.9392	0.7265	0.9289	0.8062	0.9334	0.9185
Lam_ost	0.1837	0.0655	0.1241	0.0884	0.1650	0.5646
Hav_can.	0.9175	0.1479	0.9504	0.2387	0.7779	0.8124
Ost_frag	0.7434	0.8892	0.8345	0.2360	0.6766	0.6506
Res_spac	0.4616	0.7612	0.3396	0.8085	0.8807	0.0975
Non-Hav_can	0.9306	0.7559	0.9159	0.9049	0.8541	0.5110
%Ost_bone	0.9642	0.4549	0.9568	0.5689	0.9647	0.8491
%Unr_bone	0.9738	0.5826	0.9671	0.8506	0.9815	0.5284
%Frag_bone	0.8402	0.7227	0.8919	0.0673	0.8435	0.8663
Tot_ost_mea	0.7558	0.2168	0.7409	0.2627	0.7595	0.8794

From these results it is clear that the total number of osteons (measurable and non-measurable), the minimum Haversian canal diameter, the number of osteon fragments, the number of non-haversian canals and the average percentage of osteonal, unremodeled and fragmental bone are all reliable factors. This indicates that these variables are easy to score and thus prove useful in age estimation techniques. The variables that show to be unreliable are the average number of lamellae per

osteon and the number of resorption spaces. These two variables are difficult to score and thus may not prove useful in age estimation techniques.

The following tables, Table 4.15 to 4.16, present the formulae that yielded the best results regarding the coefficient of determination and the standard error of the estimate. Table 4.15 presents the best formula for the linear regression analysis. It can be seen that for pooled sexes the single variable that displays the best relationship with age is the total number of non-measurable osteons (Tot_ost). The single variable that displays the best relationship with age in the male sample is the average percentage of osteonal bone (%Ost_bone) and the total number of osteon fragments (Ost_frag) for the female sample. The curvilinear analysis indicates the single variable best related to age for the pooled sexes and the male sample is the total number of osteons (Tot_ost); whereas the female sample is best represented by the total number of osteon fragments (Ost_frag). Thus, it is recommended that these formulae be used for estimating age from the microscopic structure of the femur.

Table 4.15 Sex specific linear regression formula representative of the best single variable for each group (t value = students t-test, SEE = standard error of the estimate, r^2 = coefficient of determination)

Age = intercept = [variable x slope] ± SEE								
Sex	**Function**	**Variable**	**Intercept**	**Slope**	**Std. error**	**t value**	**SEE**	**r^2**
Sexes pooled	**A**	**1. Tot_ost**	37.36	0.5729	0.0818	7.005	14.16	0.2541
Male	**B**	**8. %Ost_bone**	36.66	0.3757	0.0567	6.631	13.99	0.2992
Female	**C**	**6. Ost_frag**	42.85	0.7702	0.2359	3.265	13.98	0.2238

Table 4. 16 Sex specific curvilinear (quadratic) regression formula representative of the best single variable for each group (t value = students t-test, SEE = standard error of the estimate, r^2 = coefficient of determination)

Age (years) = Intercept + slope1 (variable) + slope2 (variable)^2									
Sex	**Function**	**Variable**	**Intercept**	**Slope1**	**Slope2**	**Std. error**	**t value**	**SEE**	**r^2**
Sexes pooled	**A**	**1. Tot_ost**	30.873	1.2823	-0.0135	0.2510	5.11	13.79	0.2970
Male	**B**	**1. Tot_ost**	28.729	5.9960	-0.0191	0.2676	5.98	13.26	0.3766
Female	**C**	**4. Ost_frag**	45.310	0.0009	0.0277	0.6956	0.00	13.99	0.2422

Table 4. 17 Sex specific formula for the multiple regression analysis, representative of the best variables used in combination for each group (t value = students t-test, SEE = standard error of the estimate, r^2 = coefficient of determination)

Age (years) = Intercept + ∑ (variable x slope) ± SEE							
Sex	**Function**	**Variable**	**Intercept**	**Slope**	**Std. error**	**SEE**	**r^2**
Sexes pooled	**A**	**1. Tot_ost**	187.80	0.2994	0.1836	13.93	0.3032
		4. Ost_frag		0.0470	0.5094		
		6. Non-Hav_can		-0.3337	0.1437		
		7. %Ost_bone		-1.3993	1.3828		
		8. %Unr_bone		-1.4191	1.3815		
		9. %Frag_bone		-1.3279	1.4826		
Male	**B**	**1. Tot_ost**	155.73	0.1936	0.2132	13.31	0.4152
		2. Lam_ost		0.2598	0.7404		
		3. Hav_can		0.0219	0.1101		
		4. Ost_frag		0.4695	0.6031		
		5. Res_spac		3.5399	1.2479		
		6. Non-Hav_can		-0.3256	0.1682		
		7. %Ost_bone		-1.0060	1.3586		
		8. %Unr_bone		-1.1535	1.3570		
		9. %Frag_bone		-1.8143	1.5804		
Female	**C**	**1. Tot_ost**	96.59	0.4135	0.3595	13.96	0.2860
		6. Non-Hav_can		-0.4540	0.4631		
		7. %Ost_bone		-0.6689	0.4147		
		8. %Unr_bone		-0.5019	0.3760		

Chapter 5: Discussion

The use of the microscopic analysis of bone for the estimation of age at death has become a popular technique, stimulated by modern technical advances (Maat *et al.*, 2003; 2006). To date, the methods that have been established appear to be highly reliable for estimating age at death with standard error of estimates of less than 10 years. Several variables, including total osteons, osteon fragments, resorption spaces, Haversian canal diameter, percentage of unremodeled bone, have been used to develop regression formulae in previous years. Of these variables the total osteon count and the percentage of unremodeled lamellar bone appear to be the most highly correlated with age (Kerley, 1965; Maat, 2006). However, the accuracy of these formulae to estimate age of an individual outside the study population has been questioned (Stout, 1998 and Robling & Stout, 2000). Previous researchers alluded to the fact that the achieved results may be population specific, or may also be affected by other factors such as socio-economic status, disease, mechanical stress and sample location.

The aim of this study was to develop standards for age estimation using the histological structure of bone from a South African sample. In this study the following variables were assessed, 1) the total number of osteons (non-measurable), 2) the total number of osteons (measurable), 3) the average lamellae per osteon, 4) the average Haversian canal diameter per osteon, 5) the total number of non-haversian canals, 6) the total number of osteon fragments, 7) the total number of resorption spaces, 8) the average percent of osteonal bone, 9) the average percent of unremodeled bone and 10) the average percent of fragmental bone. The moderate correlations of these variables ranged from $r = 0.4941$ to $r = 0.5469$ and the low correlations ranged from $r = 0.0003$ to $r = 0.4731$.

In this discussion, the limitations of the study, the repeatability, as well as the results of the linear and multiple regression formulae are discussed and are compared with other samples. The comparisons and differences between the correlations and coefficient of determination from this study and those of previous studies is presented in Table 5.1 and will be discussed in detail later in the discussion. Possible reasons for the outcome of this study are also discussed.

Table 5.1 Comparison of results from previous studies from the femur and of similar variables (r = correlation coefficient, r^2 = coefficient of determination, SEE: standard error of the estimate)

Correlation and standard error of the estimate for linear regression analysis			
Authors	**Variable**	**r**	**SEE**
This study (2006)	Total osteon count (non-measurable)	0.504	13.31
Kerley (1965)	Total osteons	0.922	9.39
Kerley and Ubelaker (1978)	Osteon fragments	-	6.98
Ahlqvist and Damsten (1968)	Osteons and osteon fragments	0.965	6.71
Singh and Gunberg (1970)	Total osteons	0.945	-
Ericksen (1991)	Percentage unremodeled bone	-0.720	-
Watanabe et al (1998)	Osteon perimeter	-0.835	-
Coefficient of determination and standard error of the estimate for multivariate analysis			
Authors	**Variable**	**r2**	**SEE**
This study (2006)	9 variables used	0.415	13.93
Singh and Gunberg (1970)	All variables used	0.958	3.24
Ericksen (1991)	7 variables used	0.670	10.07
Watanabe et al (1998)	6 variables	0.948	4.88
Maat et al (2006)	Percentage unremodeled bone	0.799	10.06

5.1 Limitations of the study

5.1.1 Difficulties in slide preparation

During preparation, several factors could have had an influence on the appearance of the slides which may or may not have an affect on the results. The most influential factor was the grinding of the bone sections during preparation. Care should be taken not to grind the sections down too thinly, as the histological structures will not be visible under the microscope, which could lead to misinterpretation of the structures and thus jeopardize the results. The same applies for sections being too thick, but fortunately this may be rectified by re-grinding the slide to the appropriate thickness. In addition to the sections being too thin, some of the structures may be lost if the bone slice is not ground at a perfectly horizontal level to the sandpaper and a fair amount of cracks occur which could distort the structures (Figure 5.1).

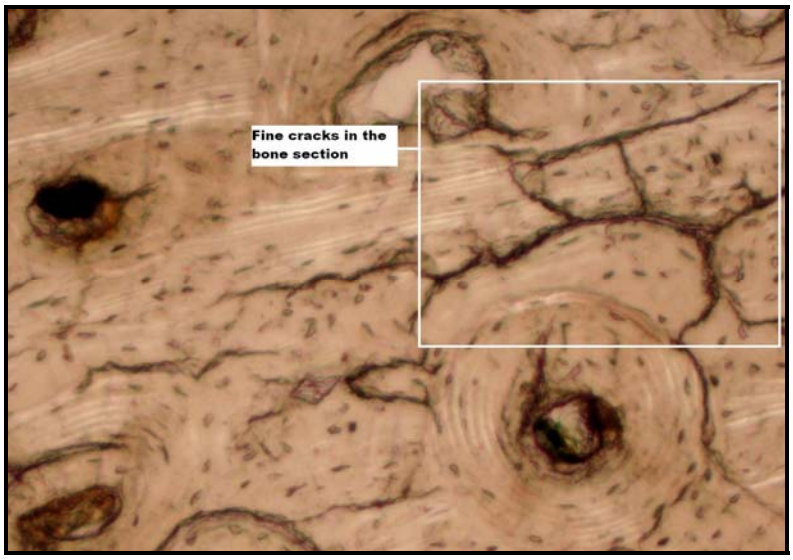


Figure 5.1 Illustration of cracks in the bone section caused by grinding

The other factor that affected the quality of the slides was the sandpaper used to grind down the sections. It was noted during the process of grinding that even though the sandpaper was of a very fine grit, there was to some degree "scratch" lines visible on the bone sections. These lines distorted the images slightly and obscure the lamellae lines, which could have affected the counting of these concentric lamellae rings. A solution to this problem was to use a much finer sandpaper to remove the major scratch lines and render a smoother more distinguishable surface. Another factor to consider when grinding the sections is that the motion in which the grinding takes place must be circular as to prevent unevenness and scratches. Sometimes air bubbles were caught under the section during the preparation. When this happened a small paperweight was placed on the cover slip directly above the bone section thus pushing the air bubble out and rendering a clear slide (Maat *et al.,* 2000). All these factors taken into consideration, the quality of slides produced in this study were adequate.

A problem encountered during the extraction of the bone sample was the undetectable micro-cracks in the shaft of the femur. When the slice was cut out and there were cracks in the shaft, the bone section split into pieces (Figure 5.2). It was prevented from further fracturing, to some degree, by applying superglue over the area where the sample was to be taken, which added some stabilization to the shaft if any cracks were present. In the case where the sections were split, they were stuck

together with superglue and prepared according to the alternative method for damaged and brittle bone sections described by Maat *et al.* (2000).

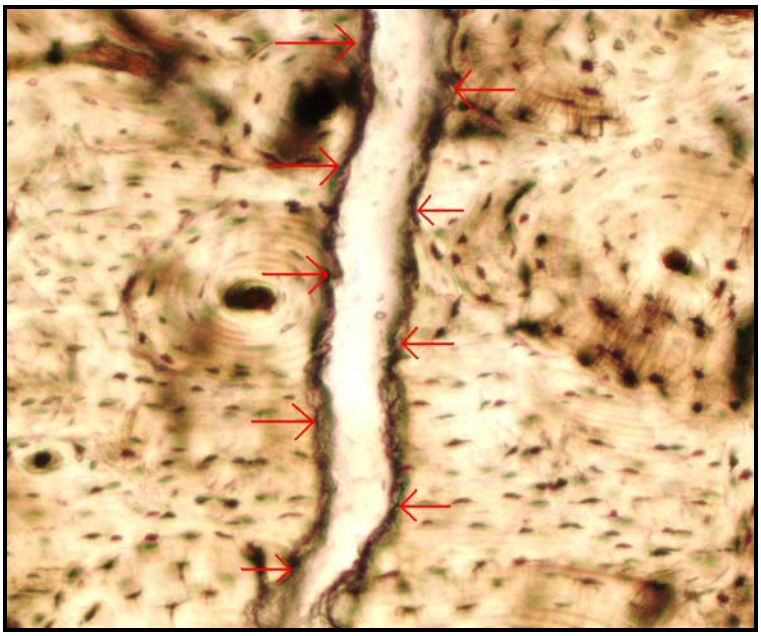


Figure 5.2 Illustration of a micro-crack (red arrows) through the bone section

While photographs seem to be modern, practical and useful for analyzing microscopic structures, it is difficult to have all the areas of the section in focus at once. Thus the photo was taken with the best focus possible, but there were always some sections that were slightly distorted especially if some areas of the slide were thicker than others (Figure 5.3). It can be seen in this figure that some of the structures are in full focus while others are slightly out of focus. The variables most affected by this are the lamellae rings and osteon fragments. Distortion makes it very difficult to distinguish between one or two lines and this could lead to an error in the results. The distortion of some areas of the photographs may lead to difficultly in distinguishing between primary and secondary osteons. The advantage of using the light microscope for direct analysis is that the focus can be adjusted as seen fit by the observer, thus allowing accurate assumptions of what structure or structures are being counted. Even though there is a problem with the clarity of the photographs used, it is a good way to keep a digital record of the bone sections. The photographs make it

effortless to send images all around the world if there is any researcher interested in analyzing them or for answering any questions about the slide.

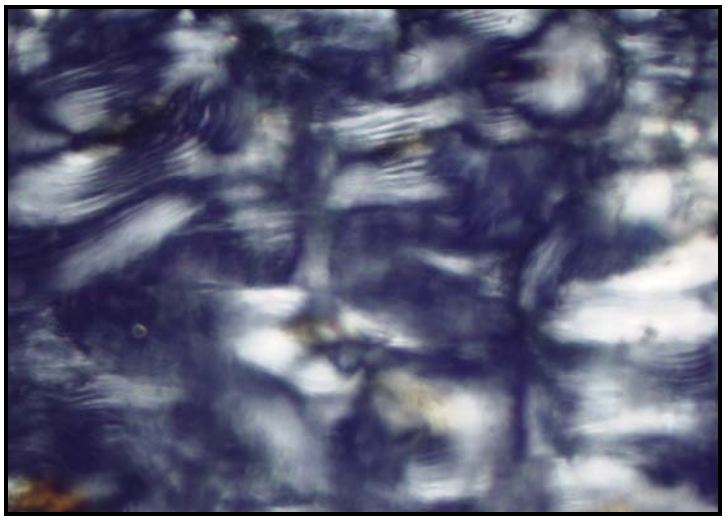


Figure 5.3 Illustration of one of the few distorted photographs indicating the difficulties encountered in some of the photos

5.2 Repeatability and reliability of the proposed method

5.2.1 Intra/interobserver error

When interpreting the definitions of the various structures described in the literature, it is difficult to identify them when a picture reference is not given. Many researchers refer to, for example, a double-zoned osteon and a Type II osteon and only give the definition without an graphical image to clearly distinguish the structures from each other or from any other structure. The most difficult structure to identify was the double-zoned osteon. It has been defined as an osteon displaying a secondary, hypercalcified cement line (Ericksen 1991; Robling and Stout 2000) but in the research conducted in this study, not one of these structures was observed. Drifting osteons are additionally difficult to identify and appear to be somewhat infrequent. Few papers or textbooks provide a graphic definition of all the structures used in histological ageing techniques and it would be an additional benefit to include such a picture reference. Type II osteons are also problematic to identify and these structures appear to be similar to double-zoned osteons making the distinction

between the two structures fairly difficult. Type II osteons are smaller osteons that have formed over the Haversian canal of a previously existing osteon. If not examined carefully it could seem that there are two hypercalcified rings and the mistake could be made to identify it as a double-zoned osteon. This is the main reason why, in this study, all the identified osteons were defined as one count so as to minimize the risk of misidentification. Ericksen (1991) obtained a moderate correlation for both the total osteon count ($r = 0.49$) and the Type II osteon count ($r = 0.55$). It is clear from these correlations that the Type II osteons appear to be more correlated with age that just the normal osteons. In this study, however, very few Type II osteons were observed. The correlation for the total osteon count is this study was $r = 0.50$, which is similar to the results that Ericksen obtained for the separate Type II and normal osteon count. From this it is should be clear that to avoid any unnecessary misidentification of Type II osteons, the two structure should be combined as one variable.

It has proved very difficult to count the lamellae rings of the osteons. The polarized light helped to some degree, but a great deal of distortion occurred and the lines could not always be distinguished from one another. This could lead to the counts being incorrect because one line was counted, where in fact it might have been two lines. This variable proved to be one of the least repeatable ($r = 0.1241$). Another factor affecting the lamellae lines is the angle at which the Haversian systems have been cut. If the osteon has been cut obliquely the lines may not be easy to see and make counting of these lines which differ from side to side very tricky. Osteon fragments also pose a concern regarding accurate counting. As explained previously, it is difficult to distinguish between primary and secondary osteon fragments especially in older individuals as there is an increase in the general number of osteon fragments. The majority of the counts that are required by these histological methods depend on the observer's experience and judgment.

5.3 Relationship and reliability between the individual variables and age

The correlations for the linear analysis (single variables) from previous studies were observed to be better than the correlations achieved in this study. This comparison is presented in Table 5.1 and will be discussed individually in the following section.

The total osteon count has been used in most, if not all, of the histological ageing techniques thus far. The reason for this is that the osteon is the fundamental structure involved with the remodeling process. All relevant authors (Kerley, 1965; Ahlqvist and Damsten, 1968; Singh and Gunberg, 1970; Ericksen, 1991; Watanabe *et al.*, 1998) found that these structures increase in number throughout life. The results of this study indicate that the total number of osteons is positively correlated with age, but only 28% of the variance in the predicted age is explained by this variable. The difference between the results of this study and those of other researchers could be attributed to factors such as sampling location, malnutrition, disease and mechanical stress. The osteon count in males was inclined to have a higher correlation with age when compared to the females, but this could be due to the small female sample compared to the relatively large male sample. Kerley (1965) did not subject his data to multiple regression analysis, but kept it to linear and curvilinear regression analysis. In his study it was found that the osteon count was highly correlated with age yielding a correlation of 0.922 (r). In Kerley and Ubelaker's (1978) revision paper, no correlation values were given, only the standard error of estimate. When compared to the SEE obtained from this study for the total number of osteons, Kerley and Ubelaker (1978) achieved a low value of ± 9.19 years whereas the present study yielded a value of ± 14.16 years. Ericksen's (1991) study yielded correlation coefficients of r = 0.49 (sex combined), r = 0.40 (female) and r = 0.58 (male) for the linear regression analysis. When compared to this study (Table 4.2) it is seen that they are generally similar to the male sample from this study being the only group with a lower correlation (r = 0.53).

The second variable used in this study (total osteon count - measurable), which is a slight alteration of the above mentioned first variable, only takes into account the osteons where the total number of lamellae are counted and the average Haversian canal diameter is measured. The aim of including this variable was to determine whether there would be a significant difference in the results between counting the osteons that were measured (canal diameter and average lamellae) and counting the total number of osteons in the field. From the results, it may be deduced that there is only a slight difference between the r-squared values and even less of a difference between the standard error of the estimates. This suggests the either one of the variables could be measured without having a significant difference in the results.

Singh and Gunberg (1970) established that there was an increase in the average number of lamellae per osteon as age increased. The correlation coefficient in this study for this variable was r = 0.294 (sexes pooled), which indicated a positive correlation. The correlation obtained by Singh and Gunberg for the average number of lamellae per osteon was r = 0.890, indicating a strong positive correlation with age. In this study, where the groups were analyzed separately it was seen that the female sample had a much lower correlation (r = 0.0185) when compared to the male sample (r = 0.3703). Either this is due to the small female sample or to the fact that female osteon development differs significantly to the male osteon development with increasing age. Singh and Gunberg did not separate male and female sample groups, since the authors accepted Kerley's note on there being no significant difference between the two. If the r-values from this study are compared to those obtained by Singh and Gunberg, it is clear that the average number of lamellae and age are not highly correlated, which could be an indication that lamellae formation is not age dependent. From this study it can be suggested that osteon size is not dependent on age due to the seemingly constant number of lamellae present through the years, which was found to be between 7 and 14 lamellae per osteon. The only factor that the number of lamellae rings depends on is their stage of development at time and age at death.

Singh and Gunberg also found that the average diameter of Haversian canals decrease with age, which would result in a negative correlation. This conclusion reached by Singh and Gunberg would also explain why the total number of lamellae increases with age. The results of this study show that the correlation is slightly positive, indicating that the Haversian canals, to some degree, become larger with age, but not significantly. The results of this study (average Haversian canal size) agree with the results obtained by Yoshino *et al.* (1994), Watanabe *et al.* (1994) in suggesting that the size of the Haversian canal does not display any significant age related changes but instead remains on average the same size throughout life. Jowsey (1966) observed that the perimeter of the osteon increases significantly with age in the femur, which is contradictory to this study, but not in the rib where it was observed that the perimeter of the Haversian canal remained the same throughout. Currey (1964) indicated in his study that the Haversian canal diameter showed a marked decrease with age which is also contradictory to this study but is in concurrence with the study conducted by Singh and Gunberg (1970). The correlation coefficient that

Singh and Gunberg achieved was r = -0.937, whereas the correlation coefficient of this study is r = 0.1377 indicating a definite difference in significance. There is no apparent explanation for this phenomenon, except that perhaps like the lamellae, Haversian canal diameter has an average size of between 30 – 70 microns per osteon, irrespective of age.

When the total number of non-haversian canals was analyzed, it was seen that the numbers tended to decrease with age. This result is consistent with the definition and formation of these canals. Kerley's (1965) and Ericksen's (1991) results indicated that there was a strong decrease in the number of non-haversian canals which is in concurrence with this study. Kerley (1965) stated that non-haversian canals were not observed in individuals over the age of 55 years, based on the deduction that all the original lamellar bone with the incorporated non-haversian canals had been completely remodeled by secondary osteons which would occupy the majority of the circumferential lamellar bone. In this study, it was found that non-haversian canals were present well beyond the age of 55 years, with some of the much older individuals displaying these canals. Although there were still non-haversian canals present in these individuals, the total number drastically declined after 60 years of age. In the male sample, non-haversian canals were not observed in the individuals who were 80 years of age, only the 82 year individual displayed five non-haversian canals. In the female sample there was only one non-haversian canal present in one of the 80 year old individuals. This indicated that even though resorption continues throughout life, not all of the lamellar bone may be replaced by secondary osteons until approximately 80 years of age. When Ericksen's (1991) results are examined, it is clear that there is no real difference between the correlation values for the male and female groups. When the males and the females of this study were compared, it was seen that there was a 5% difference between the two groups with male group tending to have a much higher total number of non-haversian canals at around the same age than the females. The best SEE obtained was in the male sample which is ± 14.02 years.

From the scatterplot (Figure 4.6) it was seen that the number of osteon fragments increased with age. These results agree with previous authors (eg., Kerley, 1965; Ahlqvist and Damsten, 1968; Ericksen, 1991) and are in concurrence with the process of bone remodeling. Ericksen (1991) achieved a high correlation value (r = 0.71) as did Kerley (r = 0.86) for the osteon fragments but this study yielded a correlation of r

= 0.3977. This indicated that the formation of osteon fragments may not be as highly correlated with age in this sample when compared to other samples. This could be due to the fact that it is fairly difficult to distinguish between primary, secondary and tertiary fragments. This may lead to unavoidable miscounts as the outlines between these fragments are not clearly distinguishable is some cases. An additional theory as to why these fragments are not highly correlated with age is the possibility of "total replacement". Osteons are formed at random areas in the lamellar bone and their sizes are also variable, so the possibility that smaller osteons could be completely replaced by a new larger osteon, leaving no trace of the former osteons behind, is not impossible. This could result in fewer actual fragments being observed, than could have potentially been present, thus influencing the total count. Two additional factors that could make this count unreliable were put forward by Singh and Gunberg (1970). Firstly they stated that most interstitial bone contains many fragments, making it difficult to count them all and secondly some osteons are not always in parallel orientation to the long axis of the bone shaft and thus secondary osteons may be cut through obliquely, giving the appearance of an osteon fragment.

The occurrence of resorption spaces throughout the sample was minimal. Only Ericksen (1991) and Yoshino *et al.* (1994) included this variable as a determinant of age from the femur and the humerus. In this study it was seen that the appearance or formation of resorption spaces does not follow a constant pattern. Resorption of bone might not be entirely dependent on age and may be influenced by other factors such as disease, nutrition, mechanical stress, etc. Although the results indicated that the incidence of resorption spaces are highly variable, it should also be considered that the location from which the sample is taken may possibly play a role. As the process of remodeling occurs, resorption cavities are formed at any location in the bone and move distally and proximally away from the origin. While the resorption cavities are being formed they are also simultaneously being filled with concentric lamellae rings. A large variation in the observed resorption spaces in this study and previous studies (eg., Ericksen, 1991; Yoshino *et al.*, 1994) indicates that this variable is not useful in estimating age.

The average percentage of osteonal bone was similar to the first variable (total number of osteons) as it included all the observable osteons in the field. The major difference was that this variable worked on percentage and included all visible areas where remodeling had occurred, regardless of whether the full osteon was present or

not. It was seen that the average percentage of osteonal bone increased with age, with relatively moderate correlations of 0.4941(sexes-pooled), 0.5469 (male) and 0.3737 (female). This was in accordance with other studies, such as Ericksen (1991) which showed correlations of 0.36 (sexes-pooled), 0.34 (female) and 0.41 for the male group. In all three instances the results obtained in this study have higher correlations when compared to those obtained by Ericksen (1991). This variable does, however, not have any advantages over the scoring of complete osteons as the osteon count (Tot_ost) showed to be slightly more correlated with age than when the percentage is taken into account.

When the scatterplot for unremodeled bone (Figure 4.7) was examined, it was seen that it decreases with age. Ericksen's (1991) results showed correlations of -0.72 (sexes-pooled), -0.78 (female) and -0.66 (male), with the female correlation being the highest out of all the variables and correlations that Ericksen achieved. The results of Maat *et al.* (2006) indicated a significant correlation with age with the r-squared value being notably high ($r^2 = 0.783$). The r-squared value attained in this study is 0.2855 and the highest correlation coefficient is 0.55 (male). Although a relationship is observed between unremodeled bone and age in this study, the correlation was markedly less than that obtained from Ericksen's (1991) and Maat *et al.* (2006), who demonstrated correlation values higher than 0.7. In fact, Maat *et al.* (2006) advocated using this histological variable alone, as a reliable indicator of age. This is reasonable as it is the only variable that is not time consuming and that does not require any complex interpretations of indistinguishable structures. The relationship between unremodeled bone and age were lower in this study, possibly due to factors such as nutrition, disease or sampling location.

Bone remodeling is a continuous process, with the result that new osteons are created over existing ones throughout life. This would imply that as an individual ages the number of old osteon fragments should also increase with age. When the scatterplot graph of osteon fragments is analyzed, it was seen that this process of remodeling occurred (Figure 4.10). The coefficient of determination in this study does not compare favourably with the correlation obtained by Kerley (1965). In Kerley's study, a correlation of 0.864 was achieved, while the correlation in this study was 0.372 (sexes-pooled) and the coefficient of determination (r^2) was 0.1582 (sexes-pooled). The lower results for this variable cannot be explained by one of the reasons given for the total number of osteon fragments, as this variable does not deal with

identifying individual primary and secondary fragments, so it does not matter whether the two are distinguished from each other. This variable relied on the identification of fragments as a whole, expressed in a percentage, thus primary and secondary fragments are not counted individually, but instead were counted together as fragmented bone. The main reason for a lower correlation could be "total replacement", which was the other reason used to explain the poor results for the total number of osteon fragments. If the osteonal bone has, to some degree, totally replaced the fragmental bone, there would be a poor correlation expected when counting the percentage of fragmental bone.

Of the ten variables examined the ones that showed the highest correlation with age were the, (1) total osteon count (Tot_ost), (2) total number of non-haversian canals (Non-Hav_can), (3) percentage of osteonal bone (%Ost_bone) and (4) percentage of unremodeled bone (%Unr_bone). It should be noted that variables (1) and (3) represent the same characteristic, but in different views. It does, however, appear that the total osteon count in better correlated with age than the percentage of osteonal bone. The remaining variables showed low or no significant correlation with age and should be excluded.

5.4 Relationship and reliability between multiple variables and age

The purpose of multiple linear regression analysis is to use the optimum combination of variables that best shows a relationship with age. In most of the studies conducted previously, the results improved when multiple variables were incorporated into an equation to estimate age at death. In this study the regression equations calculated using multiple regression analysis did not yield more accurate results than the linear regressions. The highest r-squared value was obtained when all the variables were taken into consideration (r^2 = 0.4152 - males). This implied that only 42% of the variation observed among these variables can be attributed to age. The standard error of estimate for this equation was ± 13.31 years which was the best achieved by all the regression equations. The female sample did not yield very good results, with the highest r-square value being only 0.286 when four of the ten variables were used in combination.

Kerley (1965) may not have made use of multiple regression analysis, but he stated that it is not wise to make use of only one of the variables to determine age but

instead to use a combination of as many as possible to increase the degree of accuracy. Without multiple regression analysis, Kerley achieved results that show that 92% of the variation seen in age can be explained by the total number of osteons present (Table 5.1) whereas in this study only 50% of the variation in age was explained by the osteon count (male sample).

Singh and Gunberg (1970) showed that 96% of the variation seen in age is explained by the combination of total osteon number, the average number of lamellae per osteon and the average Haversian canal diameter (males only). The present study, however, showed that the two variables, average lamellae per osteon and average Haversian canal diameter, did not have any significant correlation with age and would not be suitable to use in any regression formulae used for determining age at death. The standard error of the estimate that accompanies this correlation of Singh and Gunberg (1970) is ± 3.24 years. If compared to the results of this present study with the best SEE being ± 13.31 years, a difference of ± 10 years is noted. This major difference could, however, be due to the difference in sample size as Singh and Gunberg had a sample of 59 individuals and this study has a sample of 146 individuals. Bouvier and Ubelaker (1977) note that, as the sample size increased, so did the variability seen within the sample thus resulting in a larger margin of standard error. Ericksen's (1991) results are more comparable to results obtained in this study. The highest r-squared value achieved by Ericksen was 0.67 (sex-pooled), while the highest in this study is 0.30, indicating a 37% difference in the variance seen between the two studies. The best SEE obtained by Ericksen was ± 10.1 years (sexes-pooled) while in this study the lowest SEE is ± 13.93 years (sexes-pooled).

Watanabe *et al.* (1998) used the area, the maximum and minimum diameter and the perimeter of the perfect osteon and the Haversian canal. The authors found that the parameters set for the osteons were higher correlated (r = 0.77) with age than the parameters for the Haversian canal (r = 0.11).

Maat *et al.* (2006) stated that bone replacement is not well represented by a linear occurrence but the representation improves by a curvilinear relationship. Kerley (1965) also believed that bone replacement would be better represented by a curvilinear relationship. The results of this study showed a difference between the linear and the curvilinear correlations (Table 4.6). The range of difference observed for the various groups were as follows; sexes pooled 0.4% – 8.59%; male group 0.55% – 12.93% and the female group 0% - 2.75%. The variables that showed the

highest increase in correlation by alternatively applying curvilinear analysis were the average Haversian canal diameter (12.93% - increase in males) and the total osteon counts (measurable and non-measurable) which showed a 10.33% increase in the male group. It can be seen that there is a difference between the correlations in the study conducted by Maat *et al.* (2006) and this study for the average percentage of unremodeled bone. The r-squared value achieved by Maat *et al.* (2006) was 0.799, whereas the r-squared value for the same variable from this study was 0.248, which is approximately 55% less. When the curvilinear results were analyzed it was observed that for this variable the increase in correlation when the sexes were pooled was 1.64% making it r = 0.2648 instead of r = 0.2484. This indicated that in this study's population the percentage of unremodeled bone was highly variable and did not specifically correlate well with age. Even though the curvilinear regression analysis, in percentage, seemed to make a large difference with regards to correlation, when it was translated into actual years it makes little difference and thus the use of the linear regression formulae will be used in the future.

With regards to the multiple linear regression analysis the r-squared values and r-values of the variables observed in this study differ to some degree when compared to previous studies thus indicating that the sample used in this study does not display the highly correlated changes in bone histology as seen in previous studies (Table 5.1). It is important to note that sample size plays an important role in what the correlation and standard error of the estimate will be and it has been established that the larger the sample size the better the representation of the population will be (Allan, 1982). The smaller a sample size the less likely it is to show the true pattern of distribution for the population and small samples also tend to yield more statistical errors (Allan, 1982). In the previous studies conducted using bone histology for age estimation the sample sizes ranged between 20 and 328 individuals. Five of the relevant studies all had a sample size less than 100 individuals (eg., Alhqvist and Damsten, 1969; Singh and Gunberg, 1970; Stout and Paine, 1992; Stout *et al*. 1994; Watanabe *et al*. 1998) indicating a fairly adequate sample size according to Allan, 1982. An adequate sample size is defined as being a 100 or more individuals (Allan, 1982).

One should also bear in mind that the different variables are intercorrlelated with each other, meaning that if one variable increased another would decrease or increase. This factor may be another reason why the results of the multiple regressions are not that much improved.

5.5 Factors contributing towards variance between studies

Throughout this study, it has been shown that only 10% - 40% of the variation among the variables (either single or multiple variables) can be associated with age related changes. This is in strong contrast to other researchers (eg., Kerley, 1965; Singh and Gunberg, 1970; Ericksen, 1991; Stout, 1994, 1998; Watanabe *et al.*, 1998; Maat *et al.*, 2006) who demonstrated much stronger relationships between these histological variables and age, to the extent that in many instances 60% - 95% of the variation observed within a particular variable could be directly attributed to bone remodeling and the ageing process. With regard to inter and intra-observer error, all the recorded variables except for the average number of lamellae and the total number of resorption spaces, were shown to be consistent and hence can reliably be reproduced for age estimation techniques.

Factors present within the sample that may account for differences between this study and others include differences regarding socio-economic status (malnutrition, inadequate health care), differences in mechanical stresses between the sample groups as well as sampling location.

5.5.1 Socioeconomic status

The sample (n = 146) used in this study was of known age and racial affinity and was collected from the Student Bone Collection which is housed at the University of Pretoria. These remains were from the dissecting tables at the University of Pretoria, between 1958 and 1987. Of the sample of 146, only 16 had unknown causes of death, while the majority (27) died from some form of cancer (esophageal, liver, lung). In the remaining individuals, causes of death included diabetes (3), TB/pneumonia/other lung conditions (34), heart failure (15), liver failure (11), Pellagra and malnutrition (8) and isolated incidences of septicemia, meningitis, epilepsy, hemorrhaging, tetanus and kidney failure. These people are expected or considered to be of low socioeconomic status and this could be supported by the fact that eight of the individuals died of a nutritionally related condition (Pellagra or malnutrition). The student bone collection is mainly comprised out of unclaimed bodies received from surrounding government hospitals and the high proportion of unclaimed individuals can be attributed to the economic conditions in South Africa (L'Abbe *et al.*, 2005). Should such a person go missing or die, there may be a long delay before he is reported as missing. The

majority of individuals travel from rural to urban areas in search of work (Tal and Tau, 1983) and in many families the women and children will stay behind, while the men travel to the city to find jobs (Steyn *et al*., 1997). This situation leads to complications when the person dies it is difficult to contact family members outside of the city to come and claim the body (L'Abbe *et al.*, 2005). These unclaimed bodies are sent to the University with a relatively clear record regarding previous address, cause of death, age, race and sex. Any individuals that were not clearly recorded, regarding age, sex and race were excluded from this study. Even though the cause of death was known for the majority of the sample, it is likely that there may have been undiagnosed or untreated pathology that was not reported with the cause of death. This must be taken into account as it has been established that nutrition plays a vital role in the remodeling of bone. It has been established that nutrition, or the lack thereof, plays a major role in the remodeling of bone and thus would have an impact on histological techniques (Paine and Barrett, 2006). If individuals in this sample had previously suffered from any nutritionally related disease, the results may have been affected. Depending on the disease, different effects could be noted, for example in the case of pellagra there would have been a decrease in the formation of new bone, thus resulting in an underestimation of the individual's age, based on the histological structure of bone (Paine and Barrett, 2006). In the case of Paget's disease, an increase in new bone formation is seen, thus resulting in an overestimation of the individual's age. Paget's disease, however, is uncommon among African blacks (Tucci, 2004).

It has been established that repetitive loading leads to microcracks in the bone which can be classified as fatigue cracks (Norman and Wang, 1997). Remodeling is thus also initiated or regulated by mechanical loading or other localized, minor mechanical damage and thus should play a vital role in the remodeling of bone (Martin and Burr, 1982; Martin, 2002). The more mechanical stress that is applied to the bone the greater the incidence of microcracks and thus the higher the rate of bone remodeling (Ruff and Hayes, 1982; Martin, 2002). Martin and Burr (1982) explained that microcracks would eventually, by their course through the bone, separate the Haversian system from the surrounding lamellar bone. This separation would cause a physiological response to repair this damaged system and thus a new Haversian system would be formed to strengthen and replace the damaged Haversian system. Norman and Wang (1997) conducted a study whereby they established that microdamage appears to be more significant in females than in males and the

incidence of microcracks is greater in the femur than the tibia. The authors also established that microdamage density increases significantly with age. Martin (2002) clearly stated that although bone remodeling may be initiated by factors such as disease, disuse and trauma, virtually all cortical bone remodeling is the result of microdamage leading to microcracks. In this study high numbers of osteons were observed in the younger groups, with only a few young individuals having few if any osteons present in the lamellar bone. This could imply that these individuals experienced extreme mechanical labour such that would be expected of people working in situations that required manual labour, eg, mining. It should therefore be noted that previous working history as well as medical history of the sample is important.

5.5.2 Type of bone and sampling location

Various researchers found different results depending on which bones were analyzed (Table 5.2). It has been shown that the cranium (Cool *et al*., 1995; Curtis, 2003) demonstrated low correlations for the occipital bone (r^2 = 0.44) and frontal bone (r^2 = 0.276) respectively, whereas the mandible (Singh and Gunberg, 1970) appeared to yield the highest r-squared value (r^2 = 0.979). The other bones (tibia, clavicle, humerus, fibula, rib and ulna) have higher coefficients of determination (r2 = 0.67 – 0.98) when compared to the results obtained from the femur (r^2 = 0.42 – 0.95). The two studies that tend to have the lowest coefficients of determination are that of Cool *et al.* (1995) and Curtis (2003). Cool *et al.* used the occipital bone (r^2 = 0.44) and Curtis used the frontal bone (r^2 = 0.276). The reason the femur was specifically chosen for this study is that it is more likely to survive and remain fairly undamaged after death (Maat *et al.*, 2003).

The specific area from which the bone sections are sampled also plays an important role in the accuracy and consistency of the results. The discrepancy observed among these various bones may be attributed to the sampling location. According to Pfeiffer *et al.* (1995) circumferential and radial sampling locations are important factors when applying predictive equations based on cortical remodeling. It was established that the areas that display the most variability with regard to percentage of bone remodeling, are the anterior areas of the femur. The authors also found that in the endosteal region of the bone, a greater percentage of remodeled bone

was seen. Pfeiffer *et al.* (1995) also states that there is more variability observed along the anatomical axis of the bone when compared to the mechanical axis.

Table 5.2 Correlations (highest) found in studies using bones other than the femur (r – correlation coefficient, r^2 – coefficient of determination)

Author	Bone	Variable	Correlations
Kerley, 1965	Fibula	1) Number intact osteons, 2) Osteon fragments, 3) Non-haversian canals, 4) Unremodeled bone	r = 0.974
	Tibia		r = 0.947
Singh and Gunberg, 1970	Mandible	1) Total osteons, 2) Average lamellae per osteon, 3) Average Haversian canal diameter	r^2 = 0.979
	Tibia		r^2 = 0.964
Thompson, 1978	Tibia	19 variables (Thompson, 1978)	r^2 = 0.669
	Humerus		r^2 = 0.827
	Ulna		r^2 = 0.724
Stout & Paine, 1992	6th Rib	Sum of intact & fragmentary osteons per mm2 (OPD)	r^2 = 0.721
	Clavicle		r^2 = 0.699
	Clavicle + Rib		r^2 = 0.776
Yoshino *et al.*, 1994	Humerus	Density of 1) secondary, 2) type II, 3) double-zonal & 4) low density osteons, 5) osteons fragments, 6) resorption spaces, 7 - 8) mean & total Haversian canal area, 9 - 10) mean & total osteon area	r = 0.904
Cool *et al.*, 1995	Occipital	1) Primary osteons, 2) Secondary osteons, 3) Secondary osteon fragments, 4) Unremodeled lamellar Bone	r^2 = 0.44
Stout *et al.*, 1996	4th Rib	Sum of intact & fragmentary osteons per mm2 (OPD)	r^2 = 0.693
Curtis, 2003	Frontal bone	1) Osteon population density, 2) thickness of external table, 3-4) Mean perimeter & area of osteon, 5-6) mean minimum & maximum osteon diameter, 7-8) mean perimeter & area of Haversian canal, 9-10) mean minimum & maximum Haversian canal diameter, 11) osteon count, 12) "good" osteon count, 13) percent of "good" osteon density, 14) percent of "good" osteon area	r^2 = 0.276

In this study the bone samples were taken from the anatomical axis with the location of the sample being directly opposite the linea aspera. The main reason why the less variable mechanical axis was not used in this study was, as Pfeiffer explains,

much to invasive considering the sample used in this study it involves making a complete cross-section of the bone.

Chapter 6: Conclusion

This study illustrates that variation in certain microscopic structures of bone are systematically affected by age. Using this conclusion, regression formulae were developed to aid in age estimation from unknown human remains within a South African population. The sample used in this study (n = 146) consisted out of unclaimed bodies received from government hospitals in and surrounding Pretoria. Bone sections were removed from the anterior midshaft of the femur and subsequently bone slide were made. Ten variables were observed on the microscopic sections and from these observations both linear and multiple regression analyses were conducted. The linear regression also involved analysis of the independent variables.

The highest correlation achieved for the independent variable analysis was r = 0.547 and was seen for the average percentage of osteonal bone (%Ost_bone). The best linear regression formula using single variables for the sex pooled sample was when the total number of osteons (non-measurable) was used. The best linear regression formula achieved for the male sample was when the average percentage of osteonal bone was used and for the female sample when the total number of osteon fragments was used. It is thus recommended that should this method be applied to estimate age of an unknown individual, the formulae presented in Table 4.15 should be used. These are sex-specific formula with Function A representing the sexes pooled, Function B representing the male sample and Function C representing the female sample. It is recommended that if the sex of the unknown individual is known, that these specific formula be applied to estimate age at death. These formula originated from linear regressions, as curvilinear regressions did not show to improve the results significantly.

The best results, although more tedious to perform, would be obtained by using the sex-specific regression formula with multiple variables which are presented in Table 4.17. If the sex of the unknown individual is not known, then it is recommended that Function A from Table 4.17 be applied to estimate age. Functions B and C from Table 4.17 should be applied when the sex of the unknown individual has been determined. As can be seen from the formula presented in Table 4.17, the

best results are achieved when all of the variables are used in combination with each other.

The formulae for age estimation produced by this study do not perform as well as previously established techniques (eg., Kerley, 1965; Singh and Gunberg, 1970; Ericksen, 1991; Maat *et al*., 2006). As far as repeatability goes for the proposed method of age estimation, the interobserver error for all the variables except for the average number of lamellae per osteon and the number of resorption spaces demonstrate that the methodology is reliable.

Although the technique yields a wide age estimate, this method may provide its use in cases where the skeletal remains are extremely fragmented, with few viable bones for other methods of age estimation. Even though this method may provide a wide age range, other established age estimation techniques could be useful in providing a cut off point, either a low age estimate or a maximum estimate, so as to narrow down the age range. In specific cases where there may only be a femur shaft available for analysis, the application of this technique could be warranted. It is seen that no significant differences are observed between male and female groups, thus if the sex is unknown, the same formula can be used.

This study also contributes to the understanding that some structures (resorption spaces, lamellae per osteon, Haversian canal diameter) may not be fully dependent on age. Furthermore, remodeling of bone may be affected by external factors such as disease and mechanical stress. These factors (mechanical loading, disease) cause alterations in the remodeling process of bone, thus potentially influencing histological techniques if these factors are not identified before analysis is conducted. This misidentification could result in over and under estimation of the ages of these individuals.

Future recommendations:

Future research may include the use of a bigger sample size as well as the examination of the changes in histological structure with age in various bones (clavicle, tibia, rib, etc) instead of the femur. It is also important to make sure that the sample used in future studies is well documented with regard to age, sex and population affinity. Another possibility is to test these proposed formulae on a separate sample to ascertain the accuracy and thus the applicability of this method.

It would also be interesting to conduct a study whereby specific diseases such as Pellagra, diabetes mellitus, malnutrition and possibly cancer, could be documented with their apparent physiological effect on the remodeling process of bone and thus their consequent effect on age determination.

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APPENDIX A1: Data for the total osteons, the average lamellae per osteon, the average Haversian canal diameter, the number of osteon fragments and the number of resorption spaces

Number	Age	Sex	Tot_ost	Lam_ost	Hav_can.	Ost_frag	Res_spac
2766	20	m	32	13.1	52.1	15	0
3757	20	m	0	0.0	0.0	0	0
3283	21	m	0	0.0	0.0	0	0
1510	24	m	2	7.5	94.9	0	2
2406	24	m	0	0.0	0.0	0	0
1815	25	m	11	8.1	45.7	1	1
1888	25	m	51	10.4	41.8	13	0
3838	25	m	17	9.3	34.2	1	0
2183	26	m	15	8.5	48.5	2	1
3286	27	m	9	11.4	40.7	0	0
3713	28	m	0	0.0	0.0	0	0
4627	29	m	0	0.0	0.0	0	0
1742	30	m	10	8.3	60.9	0	1
2910	30	m	15	11.8	46.3	3	0
3931	32	m	5	7.8	52.0	0	0
4846	32	m	10	9.3	49.5	1	0
2385	34	m	11	8.8	41.2	4	0
4937	34	m	16	11.7	45.6	1	0
2445	35	m	11	10.4	55.9	2	0
3566	35	m	27	11.5	48.9	10	0
4336	35	m	16	10.3	49.2	4	0
4809	35	m	2	8.0	52.6	0	0
1837	37	m	9	8.0	32.4	0	2
2080	37	m	61	8.9	32.2	13	0
3290	37	m	14	12.1	34.8	11	0
2479	38	m	4	9.3	36.3	1	3
4284	39	m	1	16.0	72.7	0	0
2397	40	m	30	9.9	42.5	6	0
3459	40	m	0	0.0	0.0	0	2
3410	41	m	18	8.8	32.4	7	0
4406	41	m	14	12.2	73.4	0	2
4340	42	m	12	8.0	47.3	1	1
2460	45	m	32	10.0	46.0	7	0
2480	45	m	37	10.4	28.4	12	0
3960	45	m	11	9.7	51.2	2	0
1899	46	m	18	9.4	41.3	5	0
2793	46	m	1	9.0	94.2	0	0
4853	46	m	25	7.4	44.1	15	0
2303	47	m	14	10.3	42.0	4	0
3095	47	m	40	9.3	43.5	14	0
1943	48	m	6	13.0	49.2	0	1
2437	48	m	16	14.2	51.4	4	0
2895	49	m	28	9.9	52.9	9	0
3156	49	m	25	11.6	38.1	8	0
3694	49	m	16	10.3	37.6	1	0

Number	Age	Sex	Tot_ost	Lam_ost	Hav_can.	Ost_frag	Res_spac
1227	50	m	42	9.2	37.7	12	0
1957	50	m	28	11.2	47.8	3	0
4656	50	m	36	9.2	45.5	11	0
4710	50	m	34	11.9	57.9	16	0
4810	51	m	26	9.5	37.4	3	0
2272	52	m	22	10.8	38.3	16	0
4369	52	m	12	12.5	69.6	1	1
2424	53	m	23	11.4	48.1	9	0
3355	53	m	31	11.5	41.0	6	0
3489	54	m	30	10.6	46.9	5	0
3830	54	m	15	11.0	52.4	3	0
2855	55	m	27	10.7	46.8	13	0
3594	55	m	37	10.7	43.2	4	0
4424	55	m	22	11.6	44.6	2	0
4839	55	m	24	11.1	75.8	4	1
2328	56	m	51	10.4	38.3	17	1
2403	57	m	53	10.7	43.6	38	0
2945	58	m	7	7.8	46.4	0	2
4263	59	m	4	8.5	74.9	1	0
4403	59	m	35	10.2	44.9	3	0
3103	60	m	38	10.8	44.9	15	0
3262	60	m	28	12.2	33.9	6	1
4816	60	m	35	10.5	34.8	8	0
2343	63	m	39	10.4	36.9	17	0
3906	63	m	35	9.7	38.2	5	0
4242	64	m	43	9.2	52.2	13	0
4654	64	m	30	11.1	33.4	5	1
4768	64	m	22	6.9	46.9	1	3
4925	64	m	34	7.8	56.3	7	0
1776	65	m	59	9.6	40.9	12	0
2529	65	m	26	10.4	60.4	16	0
4078	65	m	33	9.6	53.0	10	0
4446	65	m	34	13.9	44.0	20	0
4715	65	m	21	13.4	42.2	9	1
4801	65	m	53	8.8	34.6	15	0
3294	67	m	27	11.3	61.6	10	1
2741	68	m	17	10.6	46.6	2	0
2829	69	m	34	11.0	36.6	32	0
2320	70	m	31	11.0	51.4	8	0
2441	70	m	21	9.6	37.9	2	0
3013	70	m	26	14.5	54.8	6	0
3367	70	m	31	9.9	48.3	6	0
3615	70	m	28	10.1	51.6	3	1
3881	70	m	24	12.5	67.7	17	1
4798	70	m	29	9.6	40.8	5	0
1914	71	m	29	7.9	39.4	8	0
4470	72	m	60	10.0	39.6	16	0

Number	Age	Sex	Tot_ost	Lam_ost	Hav_can.	Ost_frag	Res_spac
4641	72	m	14	10.5	41.9	2	0
4927	72	m	46	10.7	39.8	1	0
3686	73	m	42	11.5	49.8	31	0
1790	75	m	27	8.8	41.9	5	1
3998	75	m	49	9.8	32.9	16	0
4254	75	m	40	11.1	41.1	13	0
3465	76	m	32	10.1	33.0	4	0
3021	77	m	29	12.0	35.5	5	1
4401	79	m	35	10.7	47.5	13	3
4916	79	m	15	10.0	63.3	1	9
2452	80	m	39	11.4	42.3	30	0
4770	80	m	38	9.5	51.0	22	0
1534	82	m	24	9.7	37.7	0	0
4701	19	f	31	10.1	37.4	4	0
2340	22	f	3	11.0	46.0	1	0
3011	22	f	4	8.5	59.4	0	0
3727	25	f	16	11.9	42.7	5	1
2866	33	f	14	9.0	43.4	1	0
2929	34	f	3	10.7	57.2	0	0
2643	35	f	35	11.4	33.8	15	0
3057	38	f	13	14.0	53.5	3	0
1254	39	f	47	10.1	36.8	13	0
3024	39	f	22	10.3	32.8	1	0
2652	40	f	29	11.0	43.8	21	0
2944	40	f	33	8.3	44.2	4	0
3051	40	f	22	11.1	59.7	4	1
3853	43	f	23	13.1	44.7	5	0
1828	44	f	36	8.8	40.4	9	0
2167	45	f	33	11.1	32.7	20	0
4749	46	f	8	9.0	39.6	0	1
3040	48	f	26	12.2	42.7	6	0
4486	48	f	19	8.9	39.8	3	1
1821	50	f	34	11.1	41.8	7	0
3810	50	f	31	9.8	30.0	14	0
4054	50	f	32	10.5	49.8	25	0
2864	51	f	29	9.5	46.3	12	0
2881	51	f	26	9.7	44.1	16	0
5148	54	f	23	10.6	57.9	6	1
3806	55	f	41	11.9	46.3	11	0
3349	57	f	35	9.9	40.5	17	0
2727	60	f	19	9.0	45.5	5	0
2892	60	f	15	12.6	43.3	0	0
2979	60	f	11	7.9	55.0	1	1
4307	60	f	35	11.3	75.6	3	0
4639	64	f	52	8.9	43.9	23	0
2193	65	f	12	13.5	51.7	1	1
4855	65	f	38	10.1	49.1	18	1

Number	Age	Sex	Tot_ost	Lam_ost	Hav_can.	Ost_frag	Res_spac
4237	68	f	32	10.7	37.1	16	0
2551	70	f	40	9.2	35.3	23	0
2660	70	f	5	16.0	60.4	0	0
4889	70	f	36	9.2	54.7	1	0
2006	75	f	36	9.6	41.6	36	0
3174	80	f	49	9.9	36.9	30	0
3922	80	f	50	10.3	38.7	20	0

APPENDIX A2: Data for the number of non-haversian canals, the average percentage of osteonal, unremodeled and fragmental bone and the total osteon count (measurable)

Number	Age	Sex	Non-Hav_can	%Ost_bone	%Unr_bone	%Frag_bone	Tot_ost_mea
2766	20	m	0	71.2	15.0	13.8	16
3757	20	m	74	0.0	100	0.0	0
3283	21	m	46	0.0	100	0.0	0
1510	24	m	50	1.8	98.2	0.0	2
2406	24	m	44	0.0	100	0.0	0
1815	25	m	31	12.8	86.8	0.4	9
1888	25	m	0	79.0	10.0	11.0	27
3838	25	m	19	27.8	71.6	0.6	12
2183	26	m	21	13.4	85.8	0.8	11
3286	27	m	18	17.0	83.0	0.0	7
3713	28	m	58	0.0	100	0.0	0
4627	29	m	0	0.0	100	0.0	0
1742	30	m	19	12.6	86.6	0.8	9
2910	30	m	13	23.2	76.4	0.4	10
3931	32	m	28	4.0	96.0	0.0	4
4846	32	m	24	23.4	76.2	0.4	6
2385	34	m	28	11.2	87.6	1.2	9
4937	34	m	26	24.2	74.2	1.6	9
2445	35	m	15	15.8	82.0	2.2	8
3566	35	m	1	55.2	39.0	5.8	16
4336	35	m	9	33.2	60.2	6.6	4
4809	35	m	29	3.6	96.4	0.0	2
1837	37	m	8	9.0	91.0	0.0	6
2080	37	m	10	69.0	24.2	6.8	35
3290	37	m	13	56.0	28.4	15.6	7
2479	38	m	18	6.0	94.0	0.0	3
4284	39	m	19	3.2	96.8	0.0	1
2397	40	m	7	36.4	57.2	6.4	17
3459	40	m	44	0.0	100	0.0	0
3410	41	m	26	28.0	68.2	3.8	4
4406	41	m	13	29.8	70.2	0.0	5
4340	42	m	16	26.4	73.0	0.6	5
2460	45	m	11	34.8	61.8	3.4	23
2480	45	m	7	57.0	34.8	8.2	17
3960	45	m	27	11.2	86.8	2.0	7
1899	46	m	13	29.6	68.2	2.2	13
2793	46	m	37	6.4	93.6	0.0	1
4853	46	m	3	58.2	28.8	13.0	15
2303	47	m	6	38.6	55.8	5.6	8
3095	47	m	1	66.4	25.8	7.8	23
1943	48	m	32	15.2	84.8	0.0	4
2437	48	m	12	33.6	63.2	3.2	9
2895	49	m	3	54.2	39.0	6.8	13
3156	49	m	10	37.6	56.8	5.6	14
3694	49	m	14	24.4	75.2	0.4	9
1227	50	m	3	50.4	42.2	7.4	24

Number	Age	Sex	Non-Hav_can	%Ost_bone	%Unr_bone	%Frag_bone	Tot_ost_mea
1957	50	m	14	47.4	51.0	1.6	21
4656	50	m	4	57.4	34.2	8.4	12
4710	50	m	1	71.0	16.4	12.6	14
4810	51	m	11	34.0	64.6	1.4	15
2272	52	m	1	67.2	18.6	14.2	17
4369	52	m	12	33.6	65.2	1.2	4
2424	53	m	4	68.8	23.0	8.2	12
3355	53	m	8	50.2	46.8	3.0	17
3489	54	m	4	62.4	32.4	5.2	9
3830	54	m	11	38.8	59.6	1.6	8
2855	55	m	5	47.2	43.2	9.6	19
3594	55	m	14	66.8	30.4	2.8	9
4424	55	m	5	41.0	58.0	1.0	13
4839	55	m	11	43.0	55.8	1.2	9
2328	56	m	2	74.8	11.6	13.6	27
2403	57	m	0	75.8	4.0	20.2	28
2945	58	m	26	9.0	91.0	0.0	4
4263	59	m	26	5.0	94.4	0.6	2
4403	59	m	6	53.2	44.6	2.2	14
3103	60	m	0	66.2	22.6	11.2	17
3262	60	m	5	58.8	33.6	7.6	10
4816	60	m	2	51.6	42.8	5.6	16
2343	63	m	1	64.6	21.2	14.2	20
3906	63	m	6	41.0	57.0	2.0	18
4242	64	m	2	73.2	19.8	7.0	13
4654	64	m	6	45.0	51.6	3.4	14
4768	64	m	28	19.2	80.0	0.8	11
4925	64	m	1	43.2	51.2	5.6	15
1776	65	m	2	76.6	13.8	9.6	34
2529	65	m	1	78.2	8.0	13.8	9
4078	65	m	1	69.8	21.0	9.2	16
4446	65	m	3	69.0	19.4	11.6	11
4715	65	m	5	40.6	52.0	7.4	15
4801	65	m	0	74.4	16.2	9.4	30
3294	67	m	3	50.2	43.2	6.6	13
2741	68	m	13	33.8	65.6	0.6	9
2829	69	m	0	70.2	8.6	21.2	18
2320	70	m	2	63.2	30.6	6.2	22
2441	70	m	19	22.0	77.0	1.0	11
3013	70	m	4	64.2	32.2	3.6	10
3367	70	m	5	58.4	38.4	3.2	18
3615	70	m	2	41.2	46.6	2.2	18
3881	70	m	7	75.8	12.4	11.8	8
4798	70	m	2	54.6	41.2	4.2	18
1914	71	m	13	35.4	61.6	3.0	20
4470	72	m	0	76.8	13.0	10.2	21
4641	72	m	7	20.8	75.2	4.0	6
4927	72	m	1	64.0	35.4	0.6	29
3686	73	m	1	67.6	9.0	23.4	13
1790	75	m	7	29.0	70.0	1.0	17

Number	Age	Sex	Non-Hav_can	%Ost_bone	%Unr_bone	%Frag_bone	Tot_ost_mea
3998	75	m	18	60.6	33.4	6.0	23
4254	75	m	3	58.4	32.0	9.6	19
3465	76	m	3	71.2	24.8	4.0	15
3021	77	m	9	51.6	43.6	4.8	18
4401	79	m	2	63.4	27.6	9.0	9
4916	79	m	20	37.4	62.2	0.4	7
2452	80	m	0	70.8	0.0	29.2	22
4770	80	m	0	67.8	16.6	15.6	21
1534	82	m	5	28.8	71.2	0.0	15
4701	19	f	9	56.8	41.4	1.8	15
2340	22	f	17	4.2	94.6	1.2	3
3011	22	f	27	3.0	97.0	0.0	2
3727	25	f	5	31.4	64.8	3.8	8
2866	33	f	18	32.4	66.6	1.0	6
2929	34	f	22	5.0	95.0	0.0	3
2643	35	f	1	58.8	34.6	6.6	24
3057	38	f	12	46.0	51.2	2.8	6
1254	39	f	3	74.2	17.6	8.2	33
3024	39	f	11	38.6	60.2	1.2	10
2652	40	f	0	52.2	28.0	19.8	15
2944	40	f	2	43.2	54.6	2.2	17
3051	40	f	12	43.4	51.4	5.2	9
3853	43	f	0	46.8	48.4	4.8	11
1828	44	f	17	29.0	67.2	3.8	22
2167	45	f	1	56.2	30.4	13.4	18
4749	46	f	14	11.0	89.0	0.0	6
3040	48	f	6	69.4	26.2	4.4	13
4486	48	f	8	36.8	60.4	2.8	7
1821	50	f	0	86.8	4.2	9.0	16
3810	50	f	15	58.8	35.0	4.2	13
4054	50	f	0	71.6	10.0	18.4	11
2864	51	f	1	51.4	39.2	9.4	13
2881	51	f	0	48.0	36.2	15.8	7
5148	54	f	7	43.6	53.0	3.4	7
3806	55	f	0	77.6	15.6	6.8	20
3349	57	f	0	53.6	32.6	13.8	15
2727	60	f	1	30.0	65.6	4.4	9
2892	60	f	4	37.2	61.2	1.6	8
2979	60	f	26	15.6	84.2	0.2	8
4307	60	f	1	76.0	21.8	2.2	22
4639	64	f	2	67.8	14.4	17.8	26
2193	65	f	9	23.2	75.8	1.0	12
4855	65	f	0	72.0	11.6	16.4	15
4237	68	f	3	62.0	27.8	10.2	19
2551	70	f	1	63.4	17.0	19.6	25
2660	70	f	10	10.2	89.8	0.0	2
4889	70	f	7	51.2	46.6	2.2	26
2006	75	f	0	56.4	7.0	36.6	27
3174	80	f	0	72.6	6.8	20.6	26
3922	80	f	1	73.8	12.6	13.6	26

APPENDIX B: Data for the intra- and interobserver error analysis

Appendix B.1: Results of the repeatability for the total number of osteons that was non-measurable (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	Tot_ost (Orig)	Tot_ost (Intra)	Tot_ost (Inter)
2385	34	m	11	12	20
2479	38	m	4	6	8
3410	41	m	18	19	22
1227	50	m	42	41	40
4263	59	m	4	8	8
3906	63	m	35	40	51
3294	67	m	27	30	30
3881	70	m	24	31	35
1534	82	m	24	24	33
4701	19	f	31	32	38
3727	25	f	16	17	24
3024	39	f	22	30	34
2167	45	f	33	37	41
2881	51	f	26	30	29
3806	55	f	41	44	47
2006	75	f	36	42	48

Appendix B.2: Results of the repeatability for the average number of lamellae per osteon (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	Lam_ost (Orig)	Lam_ost (Intra)	Lam_ost (Inter)
2385	34	m	8.8	7.0	5.7
2479	38	m	9.3	8.7	8.0
3410	41	m	8.8	7.6	6.0
1227	50	m	9.2	9.9	11.6
4263	59	m	8.5	7.0	4.4
3906	63	m	9.7	8.5	7.5
3294	67	m	11.3	10.2	8.4
3881	70	m	12.5	10.1	6.6
1534	82	m	9.7	9.6	11.6
4701	19	f	10.1	9.7	8.1
3727	25	f	11.9	10.8	9.4
3024	39	f	10.3	8.5	6.6
2167	45	f	11.1	8.9	9.8
2881	51	f	9.7	10.5	7.5
3806	55	f	11.9	11.1	7.7
2006	75	f	9.6	8.6	9.7

Appendix B.3: Results of the repeatability for the average Haversian canal diameter per osteon (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	Hav_can (Orig)	Hav_can (Intra)	Hav_can (Inter)
2385	34	m	41.2	42.7	43.6
2479	38	m	36.3	36.4	35.5
3410	41	m	32.4	33.6	30.8
1227	50	m	37.7	36.4	40.6
4263	59	m	74.9	69.4	55.8
3906	63	m	38.2	36.9	37.6
3294	67	m	61.6	60.4	53.3
3881	70	m	67.7	64.9	59.3
1534	82	m	37.7	37.8	34.4
4701	19	f	37.4	36.4	38.9
3727	25	f	42.7	40.7	39.1
3024	39	f	32.8	31.1	37.9
2167	45	f	32.7	31.9	29.5
2881	51	f	44.1	45.5	40.2
3806	55	f	46.3	47.4	50.1
2006	75	f	41.6	41.8	38.7

Appendix B.4: Results of the repeatability for the total number of osteon fragments (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	Ost_frag (Orig)	Ost_frag (Intra)	Ost_frag (Inter)
2385	34	m	4	4	6
2479	38	m	1	0	0
3410	41	m	7	6	17
1227	50	m	12	17	26
4263	59	m	1	1	1
3906	63	m	5	4	10
3294	67	m	10	12	14
3881	70	m	17	15	16
1534	82	m	0	3	1
4701	19	f	4	4	4
3727	25	f	5	5	18
3024	39	f	1	3	6
2167	45	f	20	17	17
2881	51	f	16	17	32
3806	55	f	11	11	18
2006	75	f	36	31	26

Appendix B.5: Results of the repeatability for the total number of resorption spaces (Orig = Original results, Intra = Intraobserver error results and Inter = Interobserver error results)

Number	Age	Sex	Res_spac (Orig)	Res_spac (Intra)	Res_spac (Inter)
2385	34	m	0	0	0
2479	38	m	3	3	1
3410	41	m	0	0	1
1227	50	m	0	0	0
4263	59	m	0	1	1
3906	63	m	0	0	0
3294	67	m	1	1	3
3881	70	m	1	1	1
1534	82	m	0	0	1
4701	19	f	0	0	1
3727	25	f	1	1	2
3024	39	f	0	0	0
2167	45	f	0	0	0
2881	51	f	0	0	0
3806	55	f	0	0	0
2006	75	f	0	0	0

Appendix B.6: Results of the repeatability for the total number of non-haversian canals (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results).

Number	Age	Sex	Non-Hav_can (Orig)	Non-hav_can (Intra)	Non-hav_can (Inter)
2385	34	m	28	25	22
2479	38	m	18	20	26
3410	41	m	26	20	23
1227	50	m	3	1	4
4263	59	m	26	24	22
3906	63	m	6	5	3
3294	67	m	3	3	2
3881	70	m	7	0	3
1534	82	m	5	7	8
4701	19	f	9	5	10
3727	25	f	5	5	5
3024	39	f	11	7	6
2167	45	f	1	0	4
2881	51	f	0	0	1
3806	55	f	0	0	0
2006	75	f	0	0	1

Appendix B.7: Results of the repeatability for the average percentage of osteonal bone (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results).

Number	Age	Sex	% Ost_bone (Orig)	% Ost_bone (Intra)	% Ost_bone (Inter)
2385	34	m	11.2	15.2	16.6
2479	38	m	6.0	7.0	8.0
3410	41	m	28.0	25.8	18.0
1227	50	m	50.4	49.4	48.6
4263	59	m	5.0	7.0	9.0
3906	63	m	41.0	46.0	45.4
3294	67	m	50.2	48.4	45.4
3881	70	m	75.8	75.4	60.2
1534	82	m	28.8	31.2	35.8
4701	19	f	56.8	54.4	53.2
3727	25	f	31.4	31.2	26.6
3024	39	f	38.6	40.6	38.2
2167	45	f	56.2	55.8	51.8
2881	51	f	48.0	49.2	35.6
3806	55	f	77.6	71.2	68.0
2006	75	f	56.4	62.6	50.6

Appendix B.8: Results of the repeatability for the average percentage of unremodeled bone (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	% Unr_bone (Orig)	% Unr_bone (Intra)	% Unr_bone (Inter)
2385	34	m	87.6	83.2	79.6
2479	38	m	94.0	92.8	92.0
3410	41	m	68.2	71.2	71.8
1227	50	m	42.2	39.4	28.4
4263	59	m	94.4	92.6	90.2
3906	63	m	57.0	52.4	49.4
3294	67	m	43.2	44.0	44.4
3881	70	m	12.4	14.2	21.8
1534	82	m	71.2	66.8	63.8
4701	19	f	41.4	43.6	43.2
3727	25	f	64.8	63.4	63.0
3024	39	f	60.2	55.8	55.2
2167	45	f	30.4	33.0	35.0
2881	51	f	36.2	35.4	32.0
3806	55	f	15.6	20.6	23.0
2006	75	f	7.0	9.6	18.6

Appendix B.9: Results of the repeatability for the average percentage of fragmental bone (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	% Frag_bone (Orig)	% Frag_bone (Intra)	% Frag_bone (Inter)
2385	34	m	1.2	1.6	3.8
2479	38	m	0.0	0.2	0.0
3410	41	m	3.8	3.0	10.2
1227	50	m	7.4	11.2	23.0
4263	59	m	0.6	0.4	0.8
3906	63	m	2.0	1.6	5.2
3294	67	m	6.6	7.6	10.2
3881	70	m	11.8	10.4	18.0
1534	82	m	0.0	2.0	0.4
4701	19	f	1.8	2.0	3.6
3727	25	f	3.8	5.4	10.4
3024	39	f	1.2	3.6	6.6
2167	45	f	13.4	11.2	15.2
2881	51	f	15.8	15.4	32.4
3806	55	f	6.8	8.2	9.0
2006	75	f	36.6	27.6	30.8

Appendix B.10: Results of the repeatability for the total number of osteons that were measurable (Orig = Original results, Intra = Intraobserver results and Inter = Interobserver results)

Number	Age	Sex	Tot_ost_mea (Orig)	Tot_ost_mea (Intra)	Tot_ost_mea (Inter)
2385	34	m	9	9	18
2479	38	m	3	3	4
3410	41	m	4	5	20
1227	50	m	24	20	28
4263	59	m	2	4	8
3906	63	m	18	16	42
3294	67	m	13	15	26
3881	70	m	8	9	27
1534	82	m	15	16	21
4701	19	f	15	12	33
3727	25	f	8	10	18
3024	39	f	10	11	32
2167	45	f	18	17	36
2881	51	f	7	8	24
3806	55	f	20	22	35
2006	75	f	27	25	35