



5-2012

Neurocranial Histomorphometrics

Lindsay Hines Trammell

University of Tennessee-Knoxville, ltrammel@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Lindsay Hines Trammell entitled "Neurocranial Histomorphometrics." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Anthropology.

Murray K. Marks, Major Professor

We have read this dissertation and recommend its acceptance:

David G. Anderson, Joanne L. Devlin, David A. Gerard, Walter E. Klippel

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Neurocranial Histomorphometrics

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Lindsay Hines Trammell
May 2012

Copyright © 2012 by Lindsay Hines Trammell
All rights reserved.

Acknowledgements

This project would not have been completed without the help and encouragement from numerous individuals. I would like to first thank my committee chair Dr. Murray Marks for his endless patience, strong direction, and steadfast support. Thank you also to my doctoral committee – Dr. David Anderson, Dr. Joanne Devlin, Dr. David Gerard, and Dr. Walter Klippel. I am grateful for all your time and advice.

Thank you to Dr. Darinka Mileusnic-Polchan and the Regional Forensic Center for graciously allowing for the procurement of bone samples for this study. Without such amenable cooperation, this project would never have been accomplished.

I greatly appreciate Dr. Ann Reed for her statistical genius. Your continuous help and input was key to the successful finish of this research.

I am indebted to Dr. Cris Hughes for aiding in my understanding of statistics and for instilling in me a stronger passion for research. Thank you for being my inspiration and my friend.

Finally, a huge thank-you to my family for their encouragement, love, humor, and good (and bad) advice. Your unfailing confidence in me has been my strongest motivation.

Abstract

Successful development of the biological profile employs a variety of traditional methods. A key disadvantage is the necessity of near complete and proper preservation of target elements to reliably estimate age and sex. Instances lacking traditional gross odonto-skeletal features force anthropologists to rely on bone or dental microscopy.

The majority of relevant histological research discusses estimating age-at-death and has focused on numerous bones, including the ribs, clavicles and mandible, but primarily the long bones. One limitation in some previous research is the insufficient attention to how biomechanical and metabolic factors affect the osteonal remodeling process in long bones and the accuracy of aging techniques. The influence of variation resulting from localized trauma, as well as the generalized effect of diet, disease, or excessive or minimal physical activity, is also important.

Studies have also discussed microstructural differences between the sexes. There is a discord in the research as to whether or not sexes should be considered separately in establishing age-at-death estimations. However, there is no evidence in the literature as to the value of using histomorphometrics to estimate sex itself.

Past histological research has focused on bones believed to be less vulnerable to environmental stressors to better estimate age (and in this study, to potentially estimate sex). This research examines the neurocranium, specifically the frontal, parietal, and temporal. Hypothetically, this region may be less affected by biomechanical remodeling factors, given fewer muscle attachments, and has rarely been utilized in age-related histological research, especially on individuals in the young to middle-aged range.

Forty-eight white male and female decedents of known age (20 through 70 years old), sex and ancestry from the University of Tennessee Knoxville Medical Center were sampled during autopsy to remove specimens from the sectioned calotte of the left frontal, parietal and temporal bones for thin-section analysis

A research light microscope and computer imaging software were used to examine slides at various magnifications; photographic series of the entirety of each thin-section was captured using a mounted digital camera attachment. Twelve sub-variables were measured. Statistical analyses found no significant differences in mean sub-variable measurements between the internal and external tables of the thin-sections. Significant differences were noted between the sexes and by bone. Therefore, sub-variable correlations with age and regression equations to estimate age-at-death were created both together and separately for males and females for each neurocranial bone.

Sex-pooled regression equations were produced for the frontal bone and the temporal bone. Sex-specific regression equations were produced for the male frontal bone, the male parietal bone, and the male temporal bone. No variables met the significance level to be entered into the regression equations for the sex-pooled parietal bone or the female frontal, parietal and temporal bones.

A discriminant function analysis assessed if the histomorphometrics could ascertain sex. This test proved to be 80% accurate at classifying females and 90% accurate at classifying males when considering all three bones in the analysis. However, when each element was considered separately, the ability to estimate sex was increasingly weaker.

Table of Contents

Chapter 1: Introduction	1
Chapter 2: Background.....	7
Bone Biology	7
<i>Bone Function</i>	<i>8</i>
<i>Bone Cells</i>	<i>10</i>
<i>Bone Modeling</i>	<i>15</i>
<i>Bone Remodeling</i>	<i>17</i>
<i>Bone Remodeling and Age</i>	<i>26</i>
<i>Bone Formation</i>	<i>29</i>
Bone Biomechanics	34
<i>Cranial Biomechanics.....</i>	<i>37</i>
Bone Histology and Anthropology	39
<i>The Study of Histology</i>	<i>39</i>
<i>Age at Death and Sex Estimations</i>	<i>41</i>
<i>Previous Research</i>	<i>43</i>
<i>Limitations</i>	<i>52</i>
<i>Neurocranium for Age and Sex Estimations</i>	<i>53</i>
Chapter 3: Materials and Methods	59
<i>Samples</i>	<i>59</i>
<i>Sample Procurement.....</i>	<i>60</i>
<i>Thin-Section Preparation.....</i>	<i>63</i>
<i>Microscopic Examination</i>	<i>65</i>
<i>Measurements</i>	<i>67</i>
<i>Statistical Analysis</i>	<i>76</i>
<i>Intra-observer error.....</i>	<i>79</i>
Chapter 4: Results.....	80
<i>Internal versus external tables.....</i>	<i>80</i>
<i>Males versus Females</i>	<i>80</i>
<i>Independence of Observations</i>	<i>82</i>
<i>Correlations of Age with Variables by Bone and by Sex</i>	<i>82</i>
<i>Stepwise Regression.....</i>	<i>85</i>
<i>Discriminant Function Analysis</i>	<i>92</i>
Chapter 5: Discussion	95
Chapter 6: Conclusions	106
References	108
Vita	126

List of Tables

Table 3.1 Age and sex distribution of sample collection.....	61
Table 3.2 Histomorphological variables	67
Table 4.1 Repeated measures ANOVA to compare the mean values for sub-variable by table (internal and external)	80
Table 4.2 Significant (p-value) differences in sub-variable means by sex by bone	81
Table 4.3 Significant (p-value) differences in sub-variable means by bone.....	82
Table 4.4 Test for independence of observations	83
Table 4.5 Correlation (p-value) of age with measurement by bone and by sex. *Significant values at alpha=0.05.....	83
Table 4.6 Sub-variable selection and related p-values for neurocranial sexing discriminant function	94
Table 4.7 Test and resulting p-values for intra-observer error	94
Table 5.1 R-values and standard errors for regression equations	102
Table 5.2 Discriminant function classification results.....	105

List of Figures

Figure 2.1: Periosteal and endosteal membranous layers. The periosteum covers the outer bone surface while the endosteum covers the internal surface of the medullary cavity.....	9
Figure 2.2: Periosteal and endosteal borders represented on this thin-section from human frontal bone. Scale in millimeters (mm).....	9
Figure 2.3: Spongy and cortical types of bone depicted in femur. Spongy bone is more porous while cortical bone is more dense.....	10
Figure 2.4: Circles indicate osteoclasts inside resorptive space. Resorption space is indicated by arrow. Light micrograph 500x magnification.....	12
Figure 2.5: Bone-building osteoblasts lining the endosteal surface are indicated by circles. Light micrograph 500x magnification.....	12
Figure 2.6: Spidery-like canaliculi connect lacunae and provide for a means of communication. Light micrograph 300x magnification	13
Figure 2.7: Depiction of an osteon in human frontal bone including the Haversian canal, and Volkmann's canals. Light micrograph 40x magnification	14
Figure 2.8: Immature woven bone. Woven bone is loosely organized with randomly oriented collagen fibers and large areas of porosity. Light micrograph 500x magnification	16
Figure 2.9: Arrow indicates the periosteal border of the external table of the parietal bone. Circumferential lamellae bone, or layers of bone, comprise the bony matrix below. Light micrograph 100x magnification.....	16
Figure 2.10: Brick-like plexiform bone in deer humerus. Light micrograph 100x magnification.	18
Figure 2.11: Primary osteonal bone in deer frontal bone. The black arrows are pointing to the vascular canals. These canals are surrounded by concentric layers of lamellae. Light micrograph 100x magnification.....	18
Figure 2.12: Secondary osteon in human femoral thin-section surrounded by cement/reversal line. Light micrograph 100x magnification	21
Figure 2.13: Frontal bone thin-section demonstrating the visible morphological differences in primary and secondary osteons. Primary osteons are smaller and are indicative of newly formed bone while secondary osteons are larger and indicative of remodeled bone. Light micrograph 100x magnification.....	22
Figure 2.14: Human femoral thin-section demonstrating the visible morphological differences in primary and secondary osteons. Light micrograph 100x magnification.....	23
Figure 2.15: Type II secondary osteons in human frontal bone are indicated by arrows. Type II secondary osteons are smaller and embedded inside of larger osteons. Light micrograph 100x magnification	25
Figure 2.16: Drifting secondary osteon in human frontal bone indicated by arrow. A drifting secondary osteon is elongated and displays a hemicyclic tail. Light micrograph 100x magnification	25
Figure 2.17: A. The solid line represents the size and shape of an infant's long bone while the dotted line demonstrates how patterned resorption and formation drifts affect the bone during growth and development. In this example the drift patterns are diametric and	

- maintain the original form as the bone grows. B. This long bone diaphyseal cross-section represents a diametric drift pattern. The arrows indicate the uniform direction of marrow cavity and cortex expansion. C. This long bone diaphyseal cross-section is demonstrative of a unidirectional drift pattern. The smaller arrows represent the direction of drift of the cortices parallel to the diameter while the larger arrow is pointing to the drift bone 27
- Figure 2.18: Zones of the growth plate marked with arrows from left to right – resting zone, proliferative zone, hypertrophic zone, ossification zone. The resting zone is comprised of mesenchymal stem cells that differentiate into full chondrocytes in the proliferative zone. The chondrocytes fully mature and stop multiplying in the hypertrophic zone and eventually die once the matrix has mineralized or ossified. Light micrograph (scale is 250 microns) . 31
- Figure 2.19: Intramembranous Ossification. An osteoblast is visible lining the edge of the bony matrix. The osteocyte is an osteoblast that has become surrounded and encased by secreted bone matrix. Light micrograph 400x magnification 31
- Figure 2.20: Anterior and lateral views of a fetal skull. Intramembranous growth occurs along these vault sutural lines as the brain and eyes rapidly expand..... 33
- Figure 2.21: Hormonal feedback loop that controls the level of blood calcium..... 35
- Figure 2.22: Illustration of Kerley’s four quadrants in the midsection of a human femur. Black circles represent the circular fields on the anterior, medial, lateral, and posterior aspects of the bone section where Kerley gathered his data. 40x magnification..... 44
- Figure 2.23: Illustration of Ahlqvist and Damsten’s (1969) modification to Kerley. The squares represent aspects of the femoral mid-section where data were gathered. 40x magnification. 46
- Figure 2.24: Thompson’s core technique illustrated on the femur. The area removed for analysis is indicated by the arrow. Adapted from: Thompson, 1980 48
- Figure 2.25: View of skull depicting core-sampling locations (sites 1, 2 and 3) for Clarke’s 1987 research. Each core was removed at an area midway between the parietal eminence and bregma, lambda, and the sagittal suture (b-bregma, l-lambda, s-sagittal suture, p-parietal eminence)..... 55
- Figure 2.26: Approximated area on the left aspect of the occipital where Cool et al. (1995) procured bone core samples for analysis 55
- Figure 2.27: Approximated locations of bone cores removed by Curtis (2003) from the left and right frontal eminences 57
- Figure 2.28: Bone cores were sectioned parallel to, perpendicular to, or at a 45-degree angle to the sagittal suture to test for differences in measurements based on orientation. The dotted line represents the sagittal suture. The solid lines represent the direction the bone was sectioned in relation to the sagittal suture..... 57
- Figure 3.1: Examples of neurocranial samples prior to embedding (cm scale)..... 60
- Figure 3.2: Age distribution of this research compared to previous research. Notice how the current study focuses on individuals under the age of fifty 61
- Figure 3.3: Lateral view of a skull showing the sections made at autopsy and approximate location of the sampled regions of the frontal, parietal and temporal (cm scale)..... 62
- Figure 3.4: Example of three embedded neurocranial samples prior to thin-section (mm scale). 63

Figure 3.5: <i>Buehler® Isomet® Low Speed Saw</i> with sample block being sectioned with a diamond wafering blade.....	64
Figure 3.6: <i>Dual Wheel Mark® V Lab Grinder and Polisher</i>	64
Figure 3.7: <i>Leica® DMRX light microscope</i> with attached digital camera.....	66
Figure 3.8: Example of how measurements of cortical thicknesses were taken on external table of the frontal bone. 1.6x/0.05 magnification.....	68
Figure 3.9: Prepared thin-section of the external table of the frontal bone. There are adipocyte-filled lumen extending from the diploe into the external table. 1.6x/0.05 magnification.....	69
Figure 3.10: Arrow indicates a complete secondary osteon in external table of the frontal bone surrounded by reversal line. 200x magnification.....	70
Figure 3.11: Complete and fragmentary secondary osteons in the parietal bone. The complete osteon (C) is visible in its entirety while the fragmentary osteon (F) is obscured by the introduction of new secondary bone via remodeling. 200x magnification.....	71
Figure 3.12: Arrows indicate some of the secondary osteons (O) and Haversian canals (HC) in this thin-section of the frontal bone. 100x magnification	72
Figure 3.13: Complete secondary osteon outlined in black via trace tool in <i>Image-Pro® Express</i> . This tool provided area and perimeter measurements. 100x magnification	74
Figure 3.14: Haversian canal outlined in white via trace tool in <i>Image-Pro® Express</i> . This tool provided area and perimeter measurements. 100x magnification	74
Figure 3.15: Maximum and minimum diameters of complete secondary osteon noted by black lines. The trace tool in <i>Image-Pro® Express</i> took these length metrics.100x magnification	75
Figure 3.16: Maximum and minimum diameters of Haversian canal noted by white lines. The trace tool in <i>Image-Pro® Express</i> took these length metrics. 100x magnification	75
Figure 4.1: Sex-Pooled Actual by Predicted Age (in years) Plot Frontal Bone. RSq refers to the coefficient of determination and RMSE is the standard error	86
Figure 4.2: Sex-Pooled Actual by Predicted Age (in years) Plot of Temporal Bone. RSq refers to the coefficient of determination and RMSE is the standard error	87
Figure 4.3: Actual by Predicted Age (in years) Plot of Male Frontal Bone. RSq refers to the coefficient of determination and RMSE is the standard error	89
Figure 4.4: Actual by Predicted Age (in years) Plot of Male Parietal Bone. RSq refers to the coefficient of determination and RMSE is the standard error	90
Figure 4.5: Actual by Predicted Age (in years) Plot of Male Temporal Bone. RSq refers to the coefficient of determination and RMSE is the standard error	91
Figure 5.1: External table views of female (28 years) frontal (left) and male (32 years) frontal (right). Note the visible size difference in the thicknesses of the external tables. 1.6x/0.05 magnification	98
Figure 5.2: Variation in osteonal appearance in male (60 years) frontal (top), parietal (center) and temporal (bottom). Note that the size and shape of the secondary osteons and Haverisan canals as well as the organization of lamellar bone varies from one both to another. 1.6x/0.05 magnification	99

Chapter 1: Introduction

Physical and forensic anthropologists and bioarchaeologists are constantly tasked with identifying unknown human remains from forensic, historic and archaeological contexts. In forensic circumstances, the anthropologist is pivotal in the development of a biological profile from which a positive identification may be established. This profile includes age estimation, sex assessment, stature estimation and “race” conclusions from the remains. Much of this determination is based on gross morphology, using the methods of Buikstra and Ubelaker (1994), but multiple victims and perimortem and postmortem circumstances can make these methods difficult.

The primary intention of this research is to develop a new aging and sexing technique that anthropologists can use to aid in the partial estimation of that biological profile. Sex estimation in adults primarily relies on conventional metric and non-metric approaches. The most reliable non-metric based research considers gross morphological features of the pelvis, namely the pubis bone (Krogman 1962; Phenice 1969; Washburn 1948). Other non-metric studies rely on the sexual dimorphism of the cranium (Buikstra and Ubelaker 1994) and of the deciduous and permanent dentition (Hillson 1996). Metric analyses to estimate sex have been developed for almost every cranial and postcranial element. FORDISC (Ousley and Jantz 2005) classifies adults by sex using a combination of craniometric variables. Other consistent metric approaches for sexing utilize the humeral head (Bass 1995; Krogman 1962; Stewart 1979) and the femoral head (Bass 1995; Krogman 1962; Pearson 1917-1919).

Age estimation in adults has also essentially and traditionally relied on gross macroscopic methodologies including morphological changes in the pubic symphysis (Gilbert and McKern 1973; Krogman and Iscan 1986; McKern and Stewart 1957; Suchey and Katz 1998; Todd 1920),

sterno-costal characteristics of the 3rd, 4th, and 5th ribs (Iskan et al. 1984; Krogman and Iskan 1986), and the auricular surface of the ilium (Lovejoy et al. 1985a; Osbourne et al. 2004). Cranial sutures have also been used to estimate age-at-death (Acsadi and Nemeskeri 1970; Meindl and Lovejoy 1985; Todd and Lyon 1924). Both the rate and progress of sutural closure on the endo- and ectocranial aspects of the skull have been proposed to be representative of and related to age (Brooks 1955; Meindl and Lovejoy 1985; Powers 1962; Singer 1953; Todd 1925).

The disadvantage of the above-mentioned sexing and aging techniques is that they usually require a near complete and well-preserved skeleton. Oftentimes the remains are fragmented, poorly preserved or too incomplete for the anthropologist to use traditional gross morphological features to estimate a biological profile. In cases where there is no clear indication of individualized traits, anthropologists have turned to bone or dental histology, or the study of microscopic tissues (Ahlqvist and Damsten 1969; Charles et al. 1989; Crowder 2009; Gustafson 1950; Kerley 1965; Kerley and Ubelaker 1978; Stout 1992).

Bone research has focused on numerous elements but primarily on long bones (Ahlqvist and Damsten 1969; Ericksen 1991; Kerley 1965; Thompson 1979). Histological age estimation techniques have also utilized, though less often, the rib and clavicle (Stout 1992) and the mandible (Drusini and Businaro 1999).

Histological analyses have proven valuable in discerning fundamental age information about individuals. In fact, bone histology has been utilized to determine age-at-death for over forty years (Crowder 2009; Crowder and Stout 2012; Kerley 1965; Kerley and Ubelaker 1978; Stout 1992). By examining the microstructural components and nuances of bone, histologists measure and quantify features such as osteon area and diameter, Haversian canal area and

diameter, percentage of osteonal bone, and lacunae or osteocyte counts (Crowder 2009; Horni 2002; Kerley 1965; Kerley and Ubelaker 1978; Stout 1992). These data have positively demonstrated age-related changes (Ericksen 1991; Keough 2007; Kerley 1965; Kerley and Ubelaker 1978; Stout and Paine 1992; Thompson 1979, 1980).

Histological analyses have not particularly focused on estimating sex as part of the biological profile. However, some research has demonstrated the need for sex-specific analyses when developing age-at-death regression equations (Curtis 2003; Ericksen 1991) while others have noted no significant differences in microscopic age changes between males and females (Aiello and Molleson 1993; Clarke 1987; Kerley 1965; Stout and Paine 1992). Studies have also noted that the mean values of specific histological variables are significantly different between the sexes possibly indicating different rates of bone modeling and remodeling (Curtis 2003; Ericksen 1991; Kimura 1992). Though past literature has not attempted to estimate sex based on histomorphometrics, the conclusion that differences in bone remodeling do exist and that sex-specific equations to estimate age are potentially more reliable than sex-pooled equations indicate the utility of such methodologies.

It has been common practice in age-estimation histomorphometric analyses to use cortical long bone. Kerley's (1965) pioneering research has been highly influential in bone histological studies over the years. His microscopic estimation of age in human bone has been utilized in subsequent studies ever since (Ahlqvist and Damsten; Crowder and Stout 2012; Crowder 2009; Tersigni 2005). However, there are limitations to Kerley's methodology as well as others who have followed procedure including Ahlqvist and Damsten (1969), Aiello and Molleson (1993), Ericksen (1991), Thompson (1979), and Singh et al. (1974). Kerley used only

the femur, tibia, and fibula, focusing on the midshaft cortices and performed all measurements on transverse sections. These elements, however, are not always available when remains are fragmentary, making Kerley's method inapplicable.

Another limitation of previous studies is neglecting to consider biomechanical factors and how these affect the remodeling processes in long bones. Tersigni's (2005) dissertation utilized Kerley's (1965) methodology to examine within-bone differences (proximal, midshaft, and distal) and to quantify histological structures throughout the shafts of long bones. Tersigni (2005) concluded that bone maintenance and remodeling vary greatly within the shaft and that if age estimates were made in areas other than the midshaft, the resulting estimate would be significantly different.

At issue as well is that these aging methods do not factor in individual variations that may result from trauma, dietary habits, disease (paleo- and clinical pathology), or excessive or minimal physical activity (Cool et al. 1995; Stout 1989). Certain metabolic processes and nutritional deficiencies affect the complex nature of bone affecting osteonal remodeling and influencing the accuracy of histological aging techniques. Scurvy, for example, results from a lack of Vitamin C. Individuals with this ailment experience a reduction in osteoid production and a subsequent weakness in connective tissue. Paine and Brenton (2006) studied pellagra and tested the histological aging methodology of Stout and Paine (1992) to determine any potential influence of the effects of this nutritionally deficient disease. They found that when the regression formulae developed by Stout and Paine (1992) were applied to individuals who had died from pellagra, age-at-death was under-estimated by 29.2 years (Paine and Brenton 2006).

Histological research has attempted to focus on bones believed to be less reflective of

environmental stressors (Clarke 1987; Cool et al. 1995; Curtis 2003). The neurocranium is hypothesized to be less affected by biomechanical remodeling influences due its structural integrity and the fact that it is a non-weight-bearing bone (Cool et al. 1995; Laval-Jeantet and colleagues (1983). The neurocranium has not often been employed in histological age research and only a few studies attempt to quantify microscopic traits of the skull (Clarke 1987; Cool et al. 1995; Curtis 2003).

The principal intent of this research is two-fold. One goal is to use the morphohistological features in the bones of the cranial vault to develop age-at-death regression equations and to establish which element is most accurate in making this estimation. Another objective is to use these same morphohistological features in the neurocranium to estimate sex and to discern which vault bone is most successful in doing so.

The current research will examine the cranial vault, specifically the frontal, parietal, and temporal bones. These three elements were chosen for multiple reasons. They are all formed via intramembranous ossification. Both the frontal and parietal have been previously discussed in relation to age estimations (Clarke 1987, Curtis 2003) so one purpose of this research is to compare findings. Additionally, there is no evidence in previous literature that the temporal bone has been examined so this research will attempt a new approach.

Four null hypotheses will be considered prior to creating regression equations to estimate age-at-death. These hypotheses are as follows:

- H_{01} : There are no significant differences in the mean values for each sub-variable in the internal and external tables.
- H_{02} : There are no significant differences in the mean values for each sub-variable between males and females.
- H_{03} : There are no significant differences in the mean values for each sub-variable between bone by sex.
- H_{04} : The observations are independent (basic assumption of regression analyses).

Based on the acceptance or rejection of each hypothesis, regression equations to estimate age-at-death will be developed as well as a discriminant function to estimate sex.

The primary data including all digital images, copies of the author's notes and metrics will be available and accessible at the Forensic Anthropology Laboratory at the Regional Forensic Center, University of Tennessee Medical Center in Knoxville, Tennessee.

Chapter 2: Background

Bone Biology

Bone is a very dynamic tissue (Carter and Beaupre 2001; Enlow 1963; Hall 2005; Martin and Burr 1989). It functions to provide mechanical support, protect vital structures, aid in hematopoiesis and maintain mineral homeostasis. It is a composite material with both organic and inorganic components including a collagen matrix comprised of hydroxyapatite crystals. Because of the make-up of bone, it is viscoelastic and can withstand biomechanical stressors and traumatic forces to a degree (Currey 1970; Evans 1973). Bone has the capacity to absorb stress and pressure prior to fracture due to extrinsic factors such as the direction of force, the magnitude of the force, the length of time force is applied, and the rate at which force is applied. Other intrinsic variables include bone stiffness, strength, and density (Currey 1970; Evans 1973; Evans and Lebow 1951).

To understand the structure and function of bone, it is necessary to grasp the types of cells involved in cortical bone modeling and remodeling. Bone modeling involves the sculpting of bone during growth and development. New bone is produced or resorbed as individuals grow and there are shifts in areas of stress and strain (Enlow 1963). Modeling incites cortical bone changes in length and in width depending on the location and function of the bone.

Bone remodeling involves the removal and replacement of old bone with new bone. Enlow (1963) discusses this remodeling process of bone growth as a systematic coordination between bone building cells – osteoblasts – and bone resorbing cells – osteoclasts. Bone remodeling occurs throughout life at differing rates, oftentimes affected by age and pathological

or inflammatory processes (Tomes and DeMorgan 1853) and results in secondary bone called a Haversian system, or osteon. Haversian systems can be representative of primary or secondary bone. These terms and processes will be elaborated upon in subsequent sections in this chapter.

Bone Function

Bone is enveloped by two membranes (Figures 2.1 and 2.2). The periosteum, a double-layered protective covering on the outer bone surface, comprises a fibrous layer of dense cartilaginous tissue surrounding an inner osteogenic layer composed of osteoblasts and osteoclasts. This layer is rich in nerve fibers, blood, and lymphatic vessels and is secured to the bone via Sharpey's fibers. The endosteum is a membranous layer covering the internal surface of the medullary cavity. Between these soft tissues lie the cortical and trabecular layers. Bone modeling or remodeling can occur on either the periosteal or endosteal surfaces (Frost 1963a, 1964; Lacroix 1973; Martin and Burr 1989; Martin et al. 1998; Ortner 1975; Recker 1983).

There two general types of human bone: compact or cortical bone and spongy or trabecular bone (Figure 2.3). The organization of cortical and trabecular bone functions partly to allow for maximum absorption of energy with minimal trauma to the bone structure itself (Martin and Burr 1989). The cortical bone is the outer layer and is relatively more hard and dense than the inner spongy bone (Carter and Beaupre 2001; Hall 2005; Martin and Burr 1989; Recker 1983). It is primarily found in varying thicknesses in the shafts, or diaphyses, of long bones, and surrounding the trabeculae.

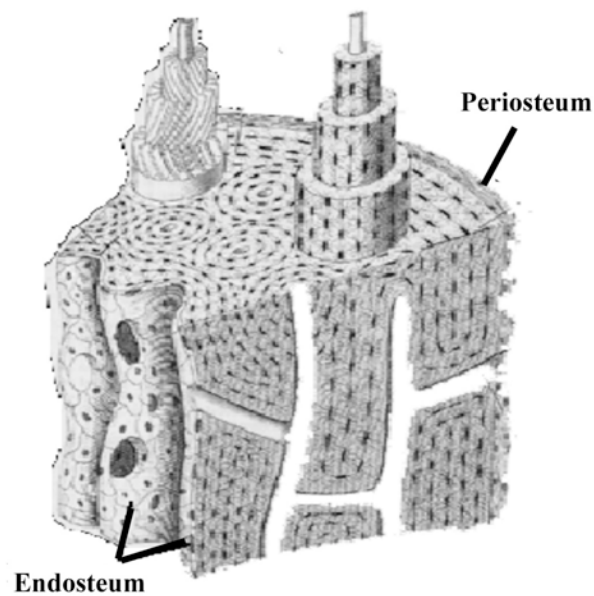


Figure 2.1: Periosteal and endosteal membranous layers. The periosteum covers the outer bone surface while the endosteum covers the internal surface of the medullary cavity.

Adapted from Junqueira et al. 1995.

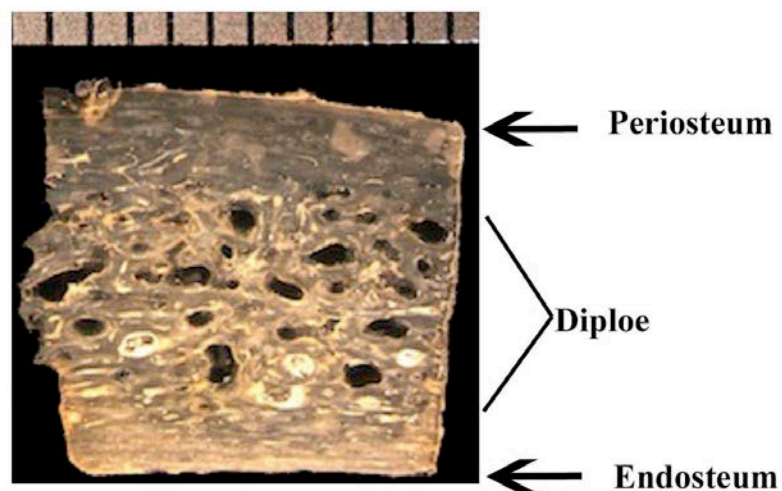


Figure 2.2: Periosteal and endosteal borders represented on this thin-section from human frontal bone. Scale in millimeters (mm).

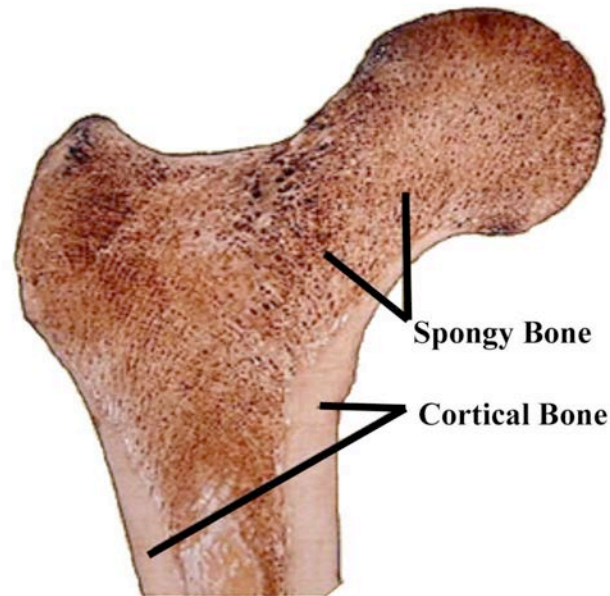


Figure 2.3: Spongy and cortical types of bone depicted in femur. Spongy bone is more porous while cortical bone is more dense.

Spongy bone is located within the medullary cavity as well as in the ends of long bones, inside the centra of vertebrae, and between cortical bone layers in the flat bones of the cranium (Carter and Beaupre 2001; Hall 2005; Martin and Burr 1989; Recker 1983). It consists of extensively connected bony trabeculae that are oriented along the lines of stress for osseous mechanical support (Trehanne 1981).

Bone Cells

There are several important cells involved in bone formation, maintenance and remodeling: osteoprogenitor cells, osteoclasts, osteoblasts, and osteocytes. Osteoprogenitor cells are derived from mesenchymal tissue. They have the ability to differentiate into a number of

different cell types. They can modify their morphologic and physiologic characteristics in response to specific stimuli (Frost 1964; Martin and Burr 1989; Recker 1983).

Osteoclasts are large, multinucleated cells that work to resorb or break down established bone matrix. They are believed to affix themselves to a bony surface and break down the bone matrix in that area via enzymal and chemical secretions (Baron et al. 1984; Heersche 1978). Osteoclastic cells resorb both cortical and trabecular bone though they are considerably more mobile in trabecular. They lie within enzymatically etched resorptive bays in the bone matrix known as Howship's lacunae (Figure 2.4).

Osteoblasts are small, single-nucleated, cuboid cells that secrete osteoid and build bone (Carter and Beaupre 2001; Frost 1964; Martin and Burr 1989; Recker 1983). Osteoid is an unmineralized organic tissue that eventually undergoes calcification and is deposited as lamellae or layers in the bone matrix. Osteoblasts are thought to originate from mesenchymal stem cells and can be the product of osteoprogenitor cells or differentiating bone lining cells. They line the periosteal or endosteal surface of bone tissue (Figure 2.5) to form bone (Baron et al. 1984; Heersche 1978; Junqueira et al. 1995).

Osteocytes are another type of bone cell. These cells are actually osteoblasts that have become surrounded and encased by secreted bone matrix. Osteocytes reside in lacunae and function by facilitating communication between bone cells and by maintaining bone integrity. These cells are linked by an extensive inter-connecting network of tunnels called canaliculi that aid in nutrient transmission and cell-to-cell communication (Carter and Beaupre 2001; Martin et al. 1998).

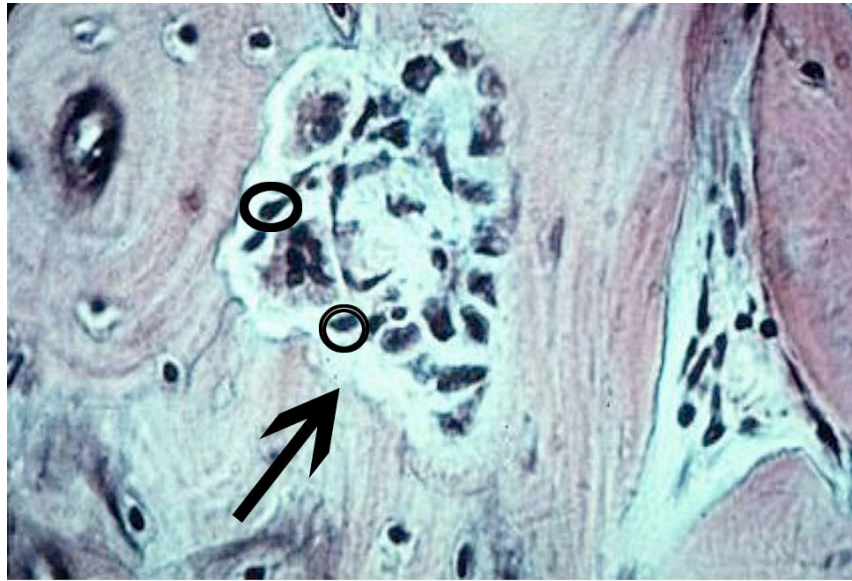


Figure 2.4: Circles indicate osteoclasts inside resorptive space. Resorption space is indicated by arrow. Light micrograph 500x magnification.

Adapted from: http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/h_frame9.html

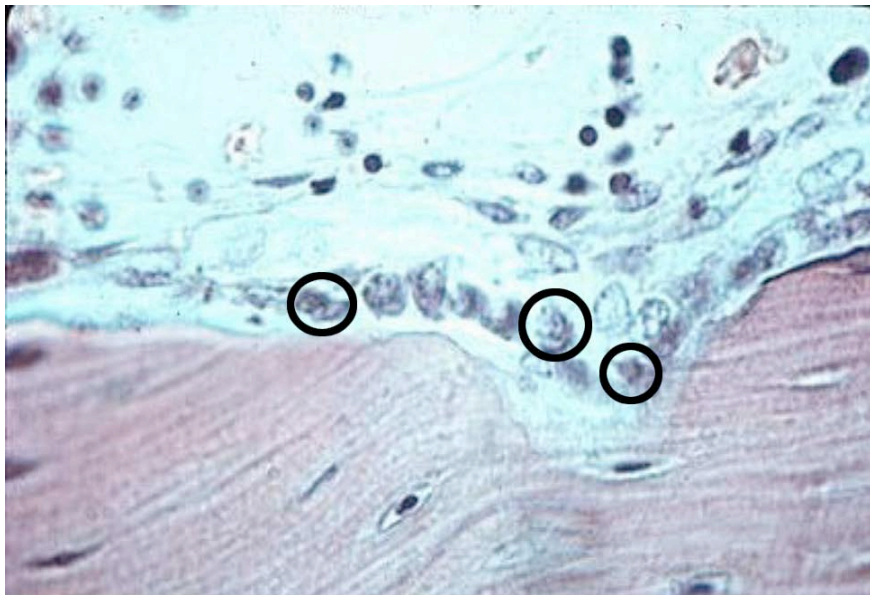


Figure 2.5: Bone-building osteoblasts lining the endosteal surface are indicated by circles. Light micrograph 500x magnification.

Adapted from: http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/h_frame9.html

Additionally within the compact bone are small spaces called lacunae that house osteocytes during life. These lacunae permeate the lamellar bone (Ardizzoni 2001). The osteocytes have finger-like extensions called canaliculi (Figure 2.6) that communicate nutrients from cell to cell. Also helping in maintain cell nourishment are Volkmann's canals which traverse the bone matrix and connect Haversian canals.

An osteon is the structural unit of compact bone (Figure 2.7). A central Haversian canal penetrates the osteon and serves as a passage for blood cells, lymph vessels and nerves. Concentric layers of lamellae composed of bone matrix surround the osteon and extend outward. Lamellar bone is comprised of mineralized osteoid. It is structured and uniform in organization and is present in both compact and spongy bone (Enlow 1962b; Frost 1964; Hall 1990; Martin and Burr 1989; Martin et al. 1998; Recker 1983).

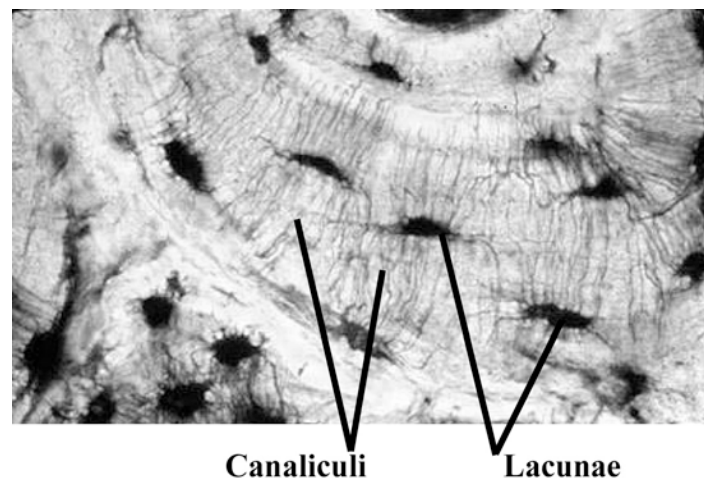


Figure 2.6: Spidery-like canaliculi connect lacunae and provide for a means of communication. Light micrograph 300x magnification.

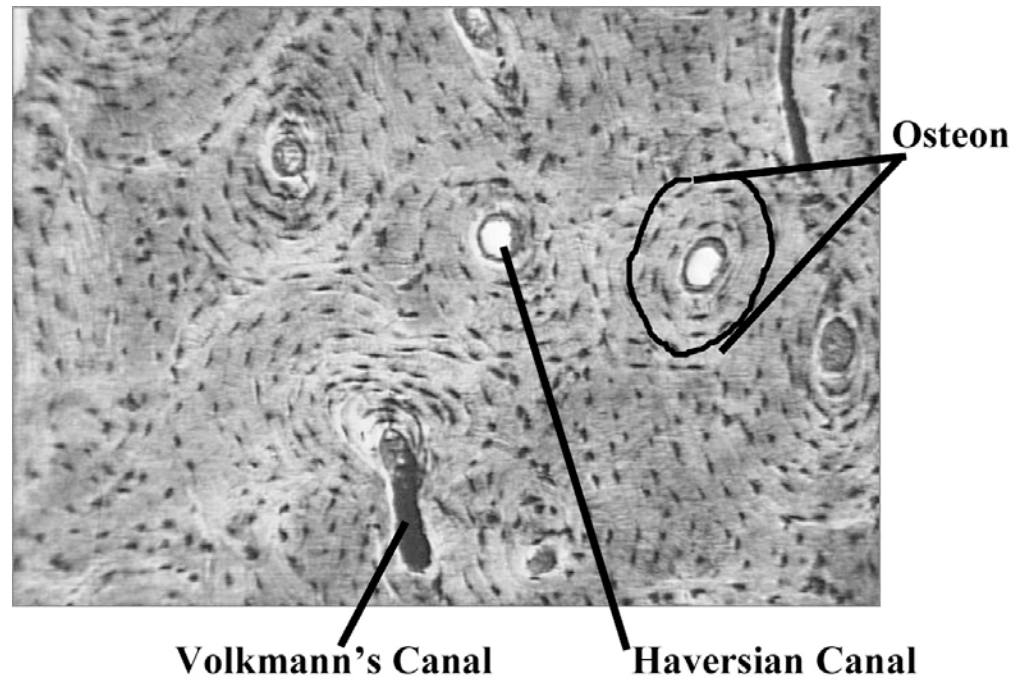


Figure 2.7: Depiction of an osteon in human frontal bone including the Haversian canal, and Volkmann's canals. Light micrograph 40x magnification.

Bone Modeling

The formation and subsequent maintenance of bone occurs during growth and development via bone modeling. Bone modeling refers to initial bone mineralization in the embryonic stage. Large areas of bone structure need to be shaped so that the bone architecture is practical and functional. Modeling can either independently build or remove bone at different rates by osteoblastic or osteoclastic activity on specific areas of bone. For example, in a long bone osteoblasts increase the diameter of the shaft by adding bone to the periosteal surface while osteoclasts remove bone from the endosteal surface (Frost 1964; Lacroix 1971; Martin and Burr 1989; Sharpe 1979).

Prior to bone modeling, tissue is comprised of loosely organized woven bone (Figure 2.8). Woven bone is immature bone with randomly oriented collagen fibers. It is temporary and eventually replaced by lamellar bone. It generally disappears when children are very young (Jaffe 1929) and is usually not found in adults unless it is in response to pathology or trauma. Woven bone can be rapidly produced to provide temporary mechanical strength during skeletal repair. It is unique in that it is the only type that can be deposited *de novo*, or without any previous cartilaginous or tissue model in place (Martin and Burr 1989; White et al. 1977).

There are three different types of primary bone that can be deposited during the modeling process (Martin and Burr 1989). The first, primary lamellar bone, is organized in a circumferential pattern between the periosteal and endosteal layers of bone (Figure 2.9).

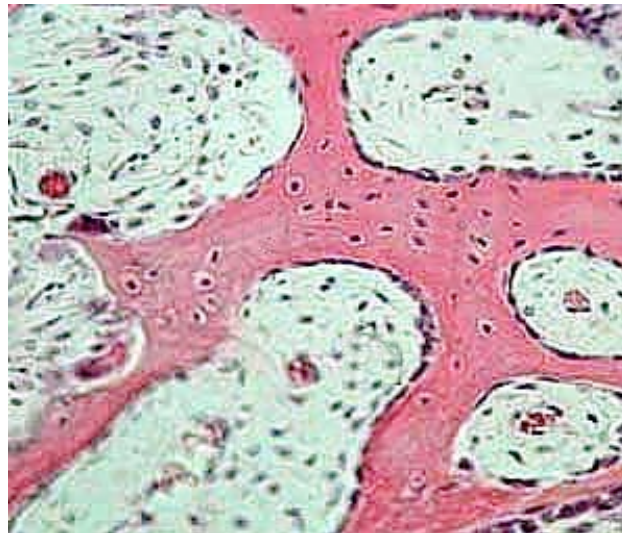


Figure 2.8: Immature woven bone. Woven bone is loosely organized with randomly oriented collagen fibers and large areas of porosity. Light micrograph 500x magnification.

Adapted from: <http://www.orthobullets.com/basic-science/9001/types-of-bone>



Figure 2.9: Arrow indicates the periosteal border of the external table of the parietal bone. Circumferential lamellae bone, or layers of bone, comprise the bony matrix below. Light micrograph 100x magnification.

The second, plexiform bone, is formed very rapidly. Since it is common for plexiform bone to develop in response to the need for increased biomechanical support, it is often seen in larger, faster growing mammals than humans including cows, sheep, or deer (Currey 2002). It is characterized by rectangular, brick-like shapes (Figure 2.10) and stems from mineral buds that grow first perpendicular and then parallel to the outer edge of bone surface (Jowsey 1966; Martin & Burr 1989).

The third type, primary osteonal bone, is comprised of circular or concentric layers of lamellae surrounding a vascular canal (Figure 2.11). These layers, the central vascular canal and osteocytes make up a primary osteon (Currey 1982; Jaffe 1929; Martin and Burr 1989). In primary bone, there is a shorter distance between osteocytes and the blood supply than in secondary bone (Dempster and Enlow 1959).

Bone Remodeling

Bone is in a constant state of turnover. Bone remodeling involves the continuous process of resorption and deposition. Remodeling is the replacement of primary bone with secondary bone. Basic multicellular units (or BMUs) are complex arrangements of cells responsible for remodeling (Frost 1964; Junqueira et al. 1998; Martin and Burr 1989). Unlike bone modeling, bone remodeling occurs as a specific site and in a consecutive order – activation, resorption, and formation (ARF). During the processes of bone remodeling, the osteoclasts and osteoblasts work sequentially to resorb and deposit new bone in response to normal turnover, biomechanical factors, hormonal changes, or increases or decreases in activity patterns. Remodeling can also refer to the repair of bone due to injury or trauma.

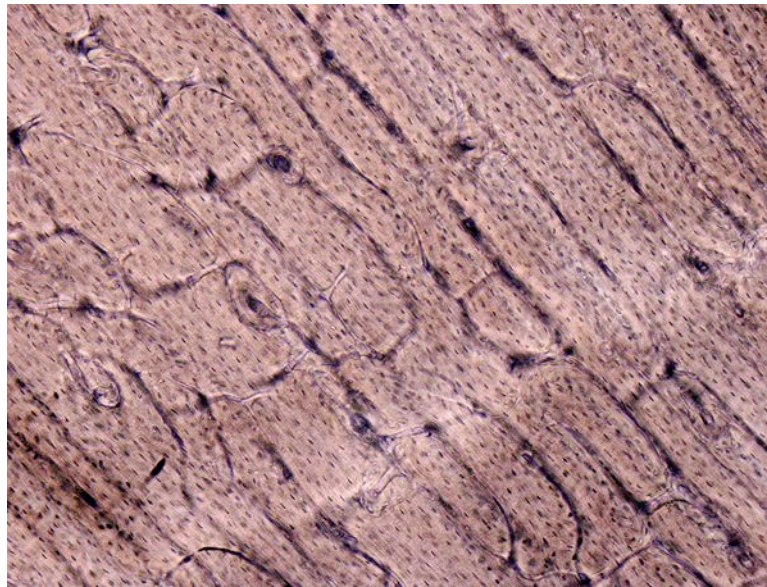


Figure 2.10: Brick-like plexiform bone in deer humerus. Light micrograph 100x magnification.

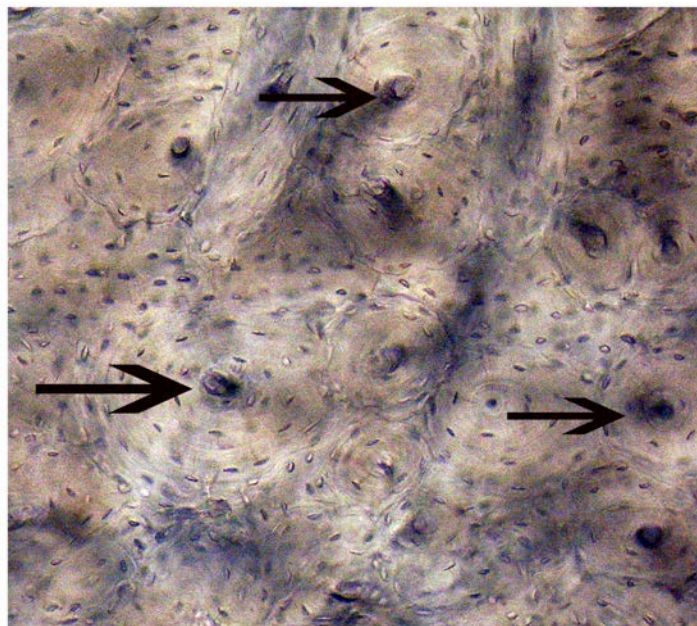


Figure 2.11: Primary osteonal bone in deer frontal bone. The black arrows are pointing to the vascular canals. These canals are surrounded by concentric layers of lamellae. Light micrograph 100x magnification.

Bone remodeling can involve the removal or resorption of older bone and the deposition of newer bone to help alleviate stress, damage, or increased strain. Martin and Burr (1989) describe “normal” bone remodeling as six successive phases. The first, activation, involves the procurement of differentiated cells from available stem cell populations. In the second phase, bone resorption, the newly derived osteoclasts begin to break down bone. The third phase, reversal, is the transitional period between the resorptive and formative stages. Osteoclastic activity converts at varying rates to osteoblastic activity. During this time secondary osteons are formed and surrounded by cement reversal lines (Frost 1964; Lacroix 1973; Recker 1983).

Bone formation is the fourth phase. Osteoblasts deposit osteoid and lay down concentric lamellae leaving a centralized Haversian canal to serve as a pathway for blood vessels and nerves. The fifth phase, mineralization, involves the calcification of the secreted osteoid. Over 60% of the osteoid becomes mineralized within the first twenty-four hours of its secretion. The final phase is quiescence. During this sixth step, osteoclasts vanish and most of the osteoblasts have become osteocytes or disappeared as well (Martin and Burr 1989).

The total amount of time required to move through these six phases is called “sigma.” Frost (1963a) coined this term with Sigma R referring to resorption time and Sigma F referring to refilling time. In cortical bone, it would take an estimated 120 days, 30 days for resorption and 90 days for formation, to move from the initial phase and resorption cavity to a completed secondary osteon (Frost 1963a; Martin and Burr 1989).

These six consecutive phases can occur in different locations in the bony matrix and at varying magnitudes and rates. An increase or decrease in the lag time between subsequent phases can affect bone structure and integrity.

Bone remodeling forms secondary bone and secondary osteons. The secondary osteon, or Haversian system, is characterized by lamellar bone surrounding a central Haversian canal with a clear cement or reversal line marking its boundary (Figure 2.12). Secondary osteons replacing primary bone are solely the result of remodeling and mature compact bone is comprised of secondary osteons.

Being able to distinguish between primary and secondary osteons is vital to this research (Figures 2.13 and 2.14). As mentioned, the primary osteon is initial bone tissue laid down on an existing bone surface and surrounded by concentric rings of lamellar bone. These concentric rings allow for the protection of the central blood vessel that passes through each osteon. If these primary osteons do not have concentric lamellae, they are called non-Haversian systems. This type of primary tissue is different from the larger secondary osteons so characteristic of human bone in its method of formation and its level of structure. It is believed that primary osteons are formed via the mineralization of cartilage while secondary osteons are the result of a remodeling process that replaces existing bone (Frost 1964; Sharpe 1979). Primary osteons are usually smaller than secondary osteons and tend to have fewer concentric rings of lamellar bone. They do not have reversal lines characteristic of secondary osteonal bone (Frost 1964; Hall 2005; Kerley 1965; Lacroix 1973; Martin and Burr 1989; Recker 1983).

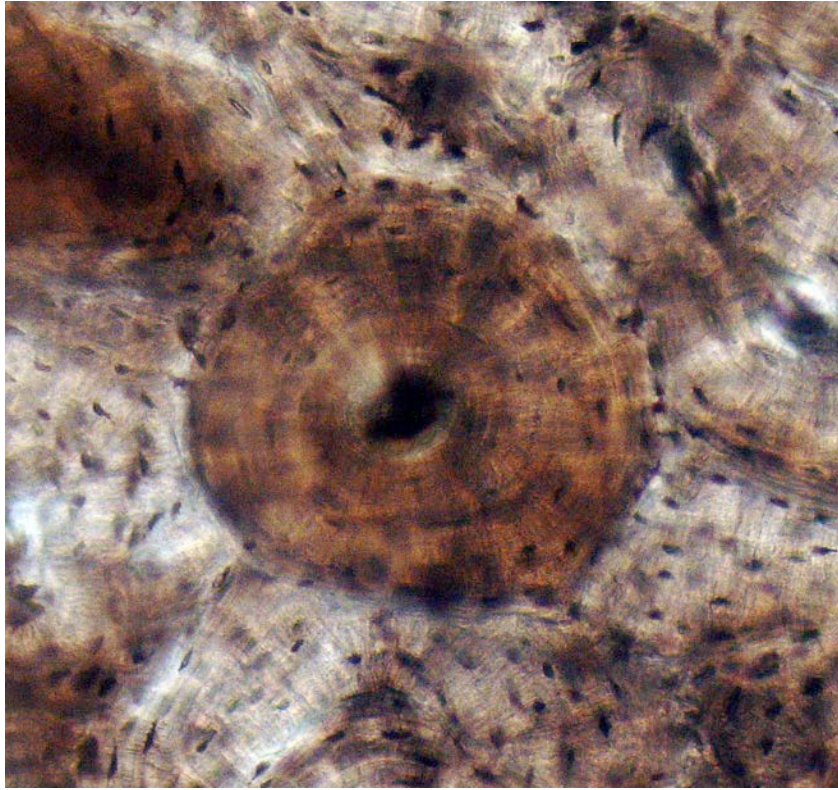


Figure 2.12: Secondary osteon in human femoral thin-section surrounded by cement/reversal line. Light micrograph 100x magnification.

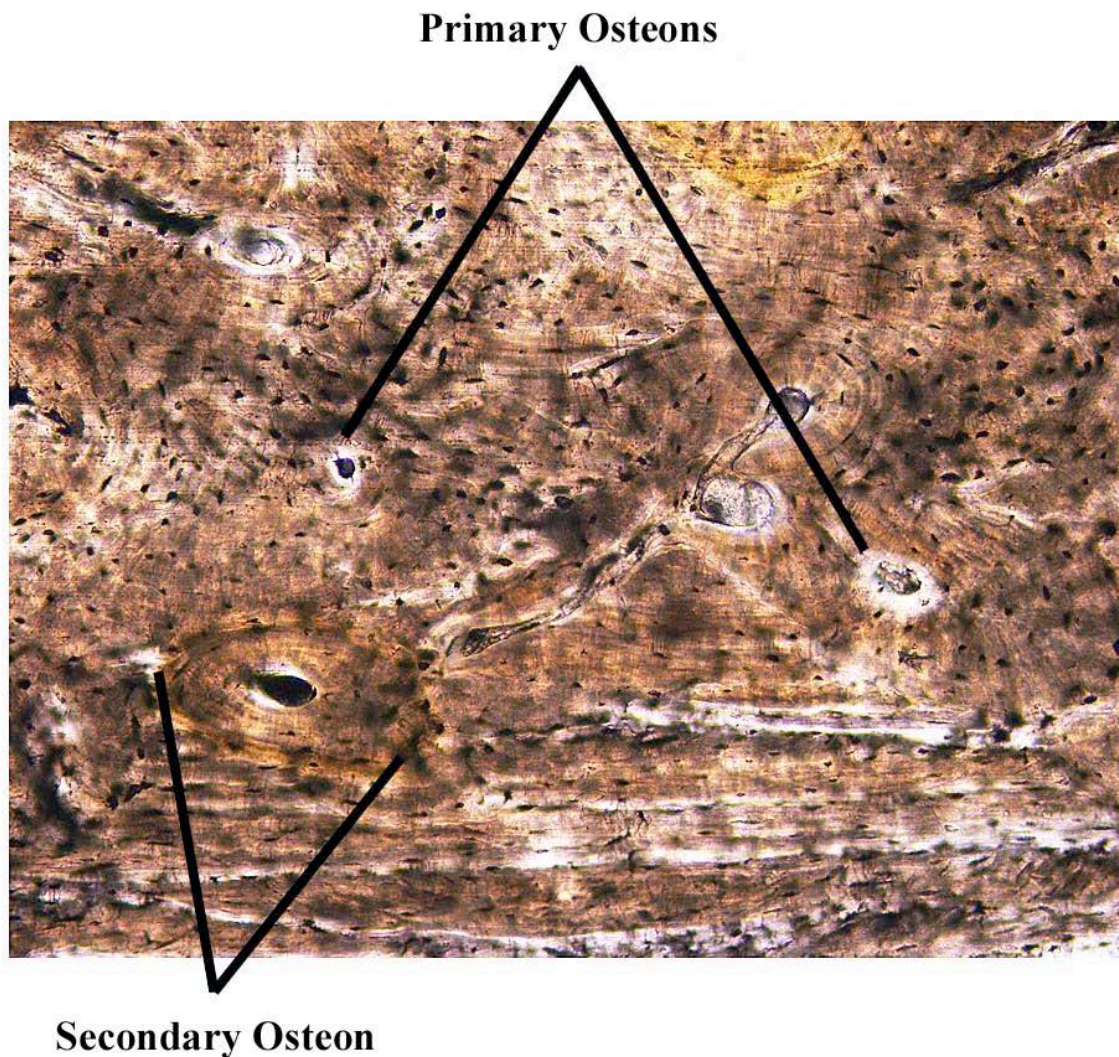


Figure 2.13: Frontal bone thin-section demonstrating the visible morphological differences in primary and secondary osteons. Primary osteons are smaller and are indicative of newly formed bone while secondary osteons are larger and indicative of remodeled bone. Light micrograph 100x magnification.

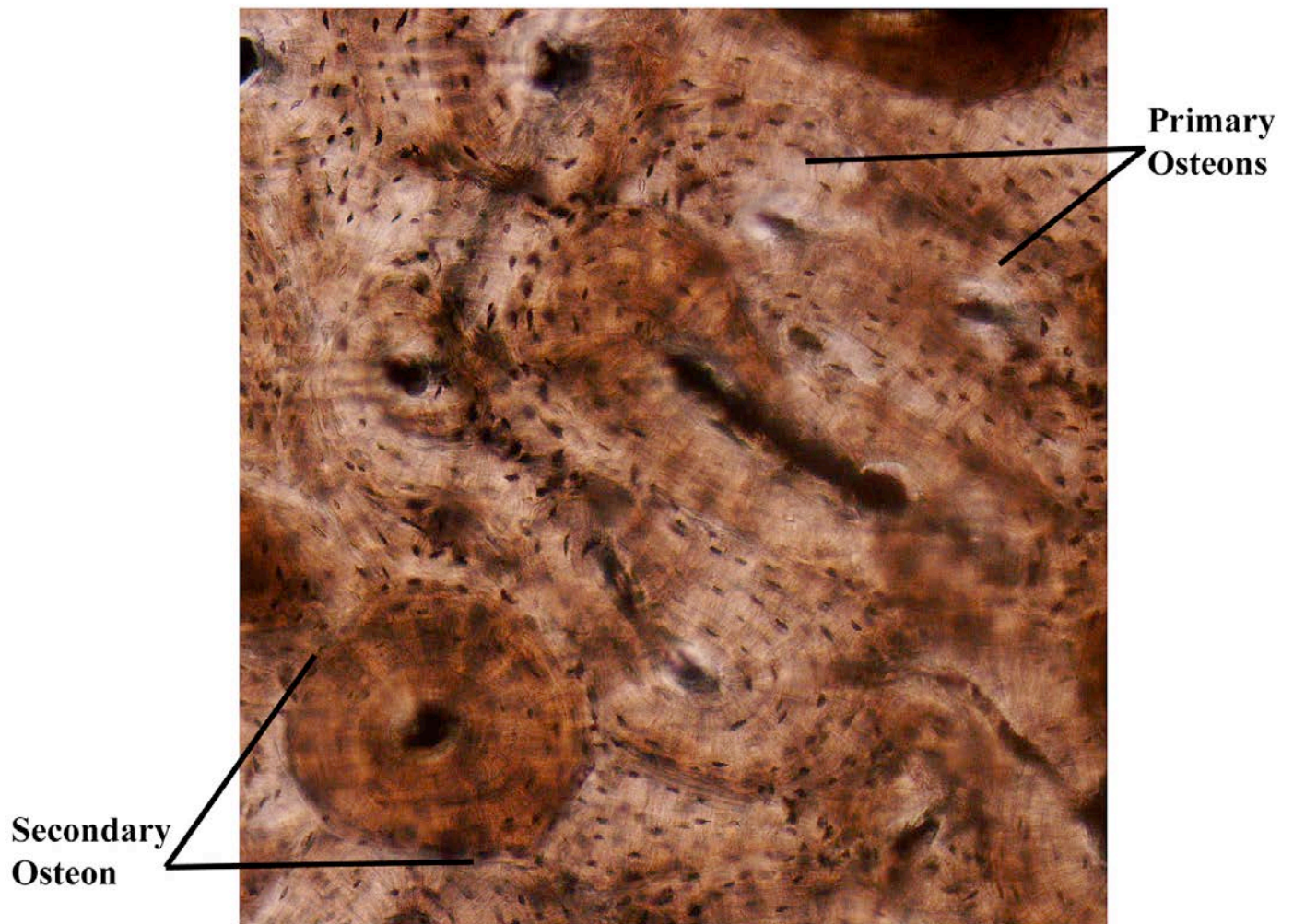


Figure 2.14: Human femoral thin-section demonstrating the visible morphological differences in primary and secondary osteons. Light micrograph 100x magnification.

There are several types of secondary osteons and each subtype shares the typical reversal line differentiating them from primary osteons. Frost (1964) describes Type I secondary osteons as common secondary osteons formed by basic multicellular units (BMUs).

Type II secondary osteons can be differentiated from Type I in several ways. Type II osteons are smaller and are embedded inside a larger osteon (Figure 2.15). These osteons form by radial remodeling of a pre-existing Haversian canal and are clearly defined by concentric lamellae and a reversal line (Robling and Stout 1999).

The drifting osteon is also a type of secondary osteon. Drifting osteons were defined first by Frost (1964) as “Haversian systems in which there is a continuous resorption on one side and continuous formation on the other.” Because of this mechanism, the drifting osteon becomes flattened on one plane and is characterized by a non-centrally located Haversian canal surrounded by concentric lamellae. Essentially a drifting osteon is present in the bone matrix when the group of cells responsible for bone remodeling travels longitudinally and transversely through the cortex resulting in an elongated osteon displaying a hemicyclic tail (Robling and Stout 1999) (Figure 2.16).

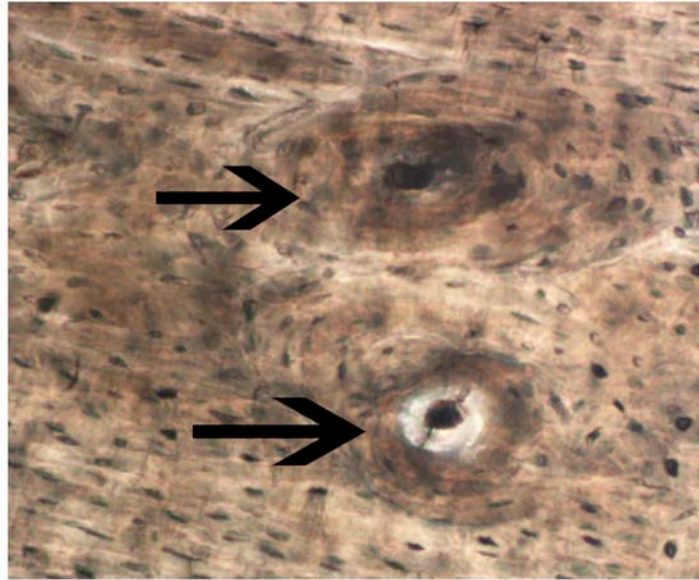


Figure 2.15: Type II secondary osteons in human frontal bone are indicated by arrows. Type II secondary osteons are smaller and embedded inside of larger osteons. Light micrograph 100x magnification.

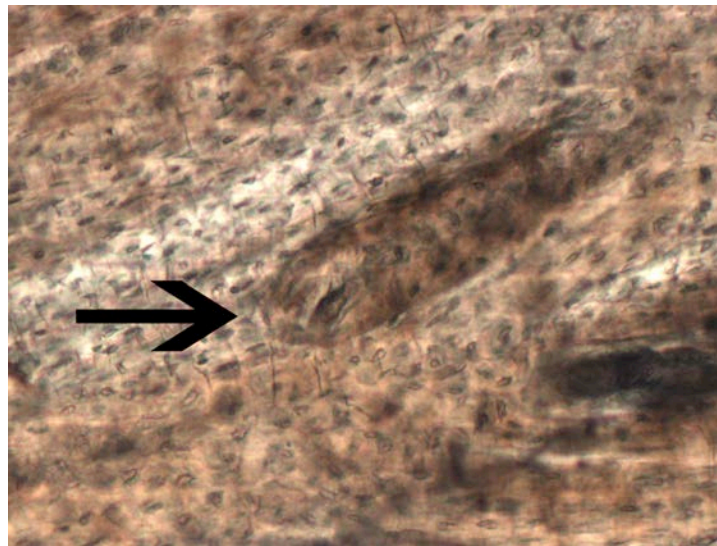


Figure 2.16: Drifting secondary osteon in human frontal bone indicated by arrow. A drifting secondary osteon is elongated and displays a hemicyclic tail. Light micrograph 100x magnification.

Bone Remodeling and Age

Bone biologists and anthropologists have recognized that with increasing age, there are concurrent microstructural changes in bone (Enlow 1963; Frost 1963a, 1964, 1987; Kerley and Ubelaker 1978; Kerley 1965; Lacroix, 1971; Martin and Burr 1989; Monro 1776; Stout 1989; Ubelaker 1989). Monro (1776) was the first to acknowledge that as an individual gets older, loss of bone occurs through expansion of the medullary cavity and related cortical thinning as well as loss of bone within the cortex itself.

Once primary bone is remodeled into secondary bone, it will always be secondary osteonal bone. Basically, secondary bone will accumulate as age increases but then ultimately be removed. Mean tissue age (MTA) is dependent upon not only this accumulation of secondary bone but also on modeling drifts, biomechanics, and hormonal and metabolic variables (Martin et al. 1980; Martin and Burr 1989; Ortner 1975). The MTA is an essential concept because it is reflective of the age of the compact bone itself. It considers the age of the individual as well as the configurations and rates of bone modeling and remodeling on all surfaces of a bone.

Modeling drifts (Amprino and Marotti 1964; Burr 1985; Jaworski 1984; Lacroix, 1971; Frost 1964, 1987) are patterned movements of the cortices of long bones during growth and development. As a bone is sculpted, the diaphysis drifts (Figure 2.17) in relation to the bone's eventual form and function resulting in some areas of the cortex appearing to have undergone more remodeling than others (Frost 1987). Cortical modeling drift can be diametric as well as unidirectional. Diametric drifts involve the uniform expansion of the diameters of the bony cortex and medullary cavity while unidirectional drifts can move cortices in a direction parallel

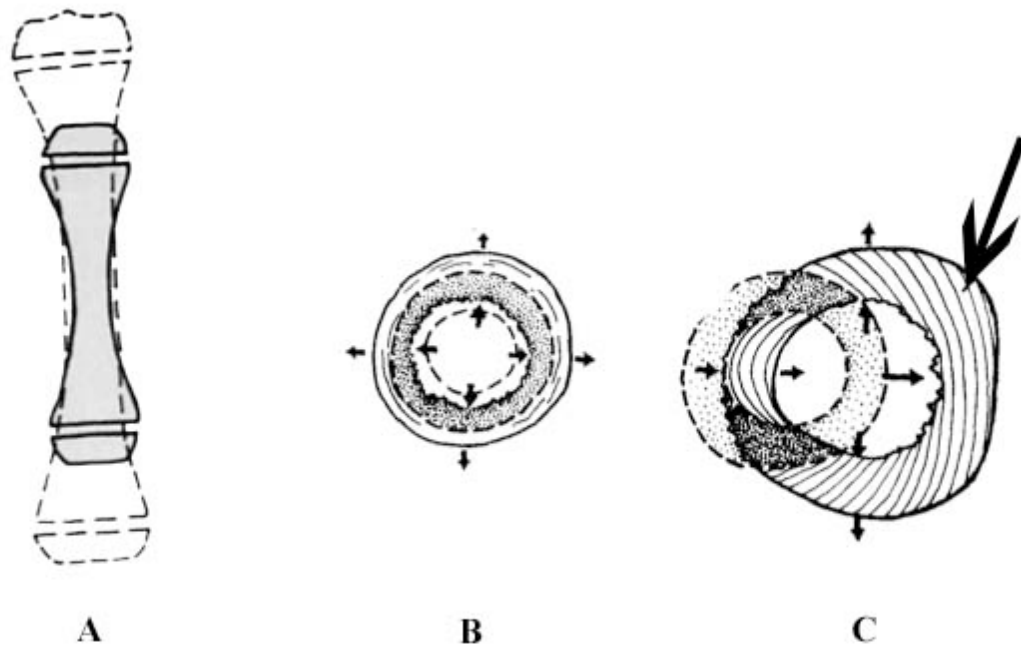


Figure 2.17: A. The solid line represents the size and shape of an infant's long bone while the dotted line demonstrates how patterned resorption and formation drifts affect the bone during growth and development. In this example the drift patterns are diametric and maintain the original form as the bone grows. B. This long bone diaphyseal cross-section represents a diametric drift pattern. The arrows indicate the uniform direction of marrow cavity and cortex expansion. C. This long bone diaphyseal cross-section is demonstrative of a unidirectional drift pattern. The smaller arrows represent the direction of drift of the cortices parallel to the diameter while the larger arrow is pointing to the drift bone.

Adapted from: Frost 1987

to its diameter (Frost 1987). These concepts are important when considering age-related changes in bone because the MTA can vary in multiple places on a single bone.

As previously mentioned basic multicellular units (BMUs) are responsible for bone remodeling. The normal aging process affects the parameters of this remodeling system, namely the activation frequency. This variable is the mean number of osteons created on an annual basis and essentially refers to how often BMUs are stimulated (Frost 1987; Martin and Burr 1989). Cortical bone turnover and the rates of remodeling in non-pathological bone are much higher when an individual is younger. For example, in human ribs, these rates reach a minimum around age thirty-five and only oscillate slightly until death, (Frost 1964; Martin and Burr 1989) potentially causing deficits in the remodeling process such as how often bone is remodeled as well as the lag time between the remodeling phases.

Bone porosity also increases with age. This is likely due to the addition of more, and larger, Haversian canals as well as resorption spaces (Atkinson 1965; Martin and Burr 1989). Smith and Walmsley (1959) found that there is a gradient of porosity across the cortex of a long bone that increases with closer proximity to the endosteal surface.

Bone Formation

As discussed earlier, the osteoblast, osteoclast, and osteocyte are the three types of bone cells that are involved in osteogenesis, the process of bone tissue formation. Ossification forms the bony skeleton of the embryo as early as the sixth week of intra-uterine life (Scheuer and Black 2000). This lasts until early adulthood contributing to bone lengthening, thickening, and continued remodeling and repair. There are two models of bone growth. The first, endochondral ossification meaning “within cartilage” creates bone by replacing hyaline cartilage-formed models. This is common in the long bones of the appendicular skeleton. The second model, intramembranous ossification, creates bone from a fibrous membrane. This is common in the bones of the neurocranium and other flat bones such those of the face and pelvis.

Endochondral ossification:

As mentioned, growth of long bones occurs as a result of endochondral ossification. This model involves a specific sequence of events beginning in the second trimester of development and continuing into the first stages of adulthood (Carter and Beaupre 2001; Martin and Burr 1989). Initial endochondral osteogenesis takes place in hyaline cartilage starting with the development of the bone collar. Once blood enters the mixture, with the surrounding perichondrium, the membrane of fibrous connective tissue covering the surface of cartilages except at the joints (Carter and Beaupre 2001; Junqueira et al. 1998; Martin and Burr 1989), the bone collar is initially formed via intramembranous ossification.

Following this process, a primary growth center appears at the rudimentary base of the diaphysis and secondary ossification centers form at the epiphyses (or ends) of the bone (Carter and Beaupre 2001; Leeson and Leeson 1981). Longitudinal growth occurs by chondrocytes in

an area near the ends of bone called the growth plate or physis. The physis serves as a boundary between the bone epiphysis and metaphysis (Martin et al. 1998). The growth plate is divided into zones: resting, proliferative, hypertrophic and provisional calcification (Figure 2.18).

Chondrocytes progress through these above-mentioned subsequent phases during their life cycle ultimately resulting in a calcified cartilage matrix (Martin et al. 1998) followed by a zone of ossification (Leeson and Leeson 1981). During this process, osteoblasts accumulate at these calcified areas to lay down bone.

Intramembranous ossification:

Intramembranous bone development occurs within the flat bones of the skeleton, including the neurocranium, the pelvis and some bones of the face. It is also responsible for the thickening of long bones undergoing endochondral growth (Carter and Beaupre 2001; Junqueira 1998). This process is seen as early as the eighth week of gestation. During this time, undifferentiated mesenchymal cells directly differentiate into osteoblasts and begin to deposit osteoid. This unmineralized bone matrix eventually surrounds the osteoblast and mineralizes (Figure 2.19). The encased osteoblasts are now known as osteocytes housed in lacunae. During bone growth, osteoblasts develop on the periosteal surface of pre-existing bone and deposit on these areas to increase bone thickness (Leeson and Leeson 1981).

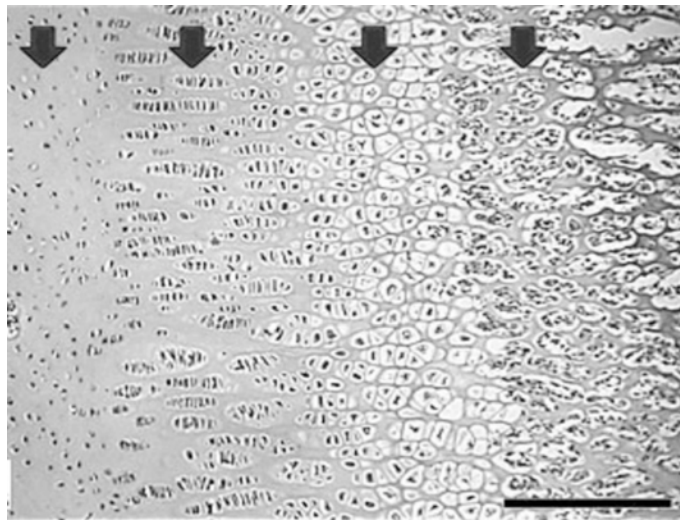


Figure 2.18: Zones of the growth plate marked with arrows from left to right – resting zone, proliferative zone, hypertrophic zone, ossification zone. The resting zone is comprised of mesenchymal stem cells that differentiate into full chondrocytes in the proliferative zone. The chondrocytes fully mature and stop multiplying in the hypertrophic zone and eventually die once the matrix has mineralized or ossified. Light micrograph (scale is 250 microns).

<http://www.kumc.edu/instruction/medicine/anatomy/histoweb/bone/bone02.htm>

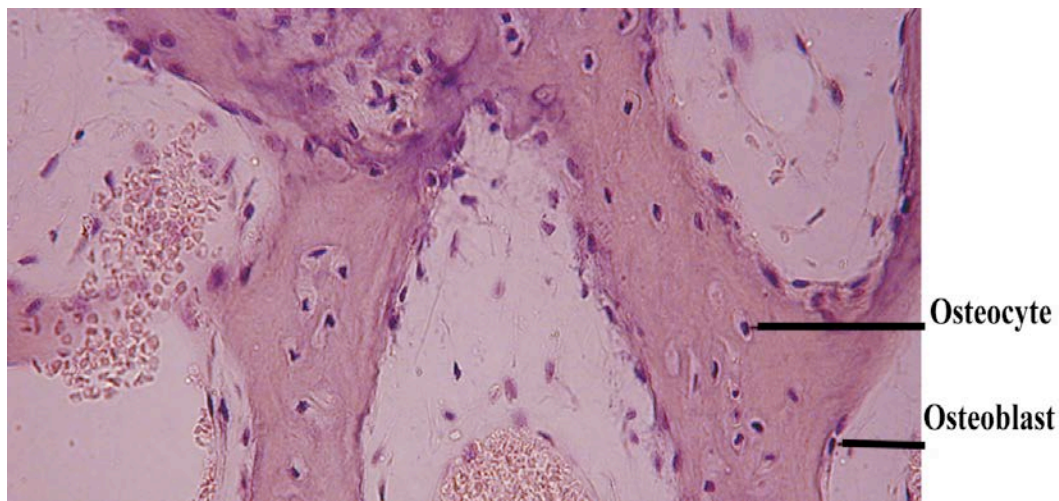


Figure 2.19: Intramembranous Ossification. An osteoblast is visible lining the edge of the bony matrix. The osteocyte is an osteoblast that has become surrounded and encased by secreted bone matrix. Light micrograph 400x magnification.

Adapted from: <http://kcfac.kilgore.cc.tx.us/kcap1/images/>

During growth and development of the cranial bone, both endochondral and intramembranous ossification occurs. The cranial base and the majority of the nose, i.e. the basal and lateral parts and the lower squama of the occipital, the petromastoid parts of the temporals, the body, the lesser wings and the medial parts of the greater wings of the sphenoid, the ethmoid, and the inferior nasal conchae, are formed via endochondral ossification (Scheuer and Black 2000). The bones of the vault, which are the focus of this research, form via intramembranous growth to protect the brain as it expands. These elements include the frontal, parietals, greater wings of the sphenoid, squamous aspects of the temporals and the squama of the occipital.

The major sites of intramembranous growth and expansion occur along the vault sutures (Figure 2.20) (Duterloo and Enlow 1970; Opperman 2000). Enlow (1986, 2000) distinguishes between growth centers and growth sites. Bone growth centers have an intrinsic growth potential, an example being the epiphysis of a growing long bone (Enlow 1986, 2000; Opperman 2000). Growth sites are secondary, adaptive areas where growth remains dormant until stimulated by an external signal. The external signal in this case is the expanding brain. As the brain expands and the cranial bases' synchondroses become longer and wider, the neurocranial sutures respond by adding intramembranously formed bone at the edges of the mineralizing fronts. This enables the sutures to remain at approximately the same width while the cranial vault increases in size in response to the growing brain (Opperman 2000). Essentially, cranial bones grow primarily via the formulation of bone tissue within the periosteum between sutures and on the external bone surface (Junqueira et al. 1998).

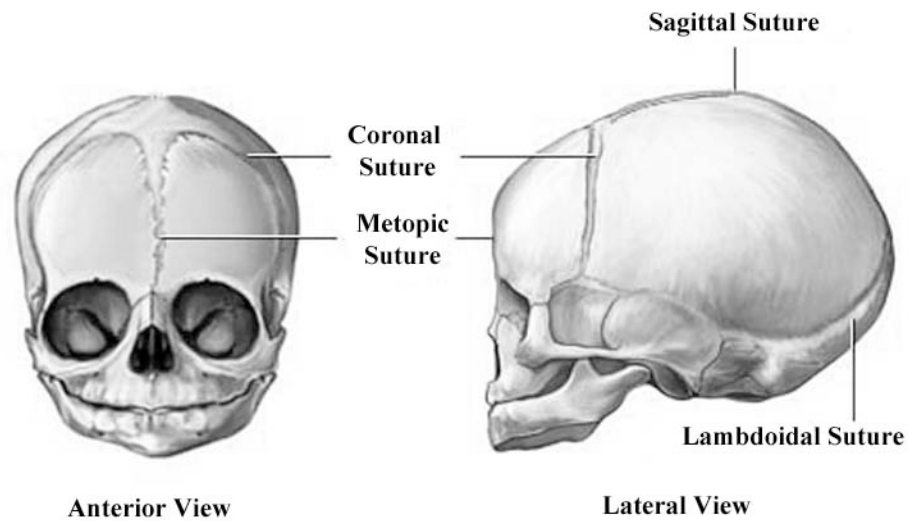


Figure 2.20: Anterior and lateral views of a fetal skull. Intramembranous growth occurs along these vault sutural lines as the brain and eyes rapidly expand.

Adapted from: <http://catalog.nucleusinc.com>

Bone Biomechanics

Bone responds to both mechanical and non-mechanical factors. The bone remodeling process is constant and is partly regulated by the body's hormonal system, a process independent of mechanical adaptation. A feedback loop (Figure 2.21) helps to maintain and control the amount of calcium ion levels in the blood – a balance that is vital to health and bone strength. To keep such a balance, the thyroid gland releases a hormone called calcitonin when the calcium ion levels in the blood reach abnormally high levels. The calcitonin stimulates the osteoblasts to deposit calcium salts into bone. Similarly, when calcium ion levels in the blood become too low, the parathyroid gland is stimulated to release a hormone called Parathyroid hormone (PTH). PTH signals osteoclastic activity to break down and resorb bone matrix to release calcium ions back into the bloodstream (Johnson 1964; Frost 1964; Lanyon 1981; Martin and Burr 1989; Stout 1992).

Aside from physiological, bone is also subject to forces and moments of varying strengths and directions (Hughes-Fulford 2004; Petrtyl et al. 1996). Once bone modeling is complete, biomechanical stressors and forces still affect the gross morphology of bone. Bone adapts to its mechanical environment and does so through ARF remodeling or damage repair. These effects will be visible at the microscopic level. Studies have attempted to observe and explain such changes (Ascenzi and Bonucci 1967, 1968; Currey 1959).

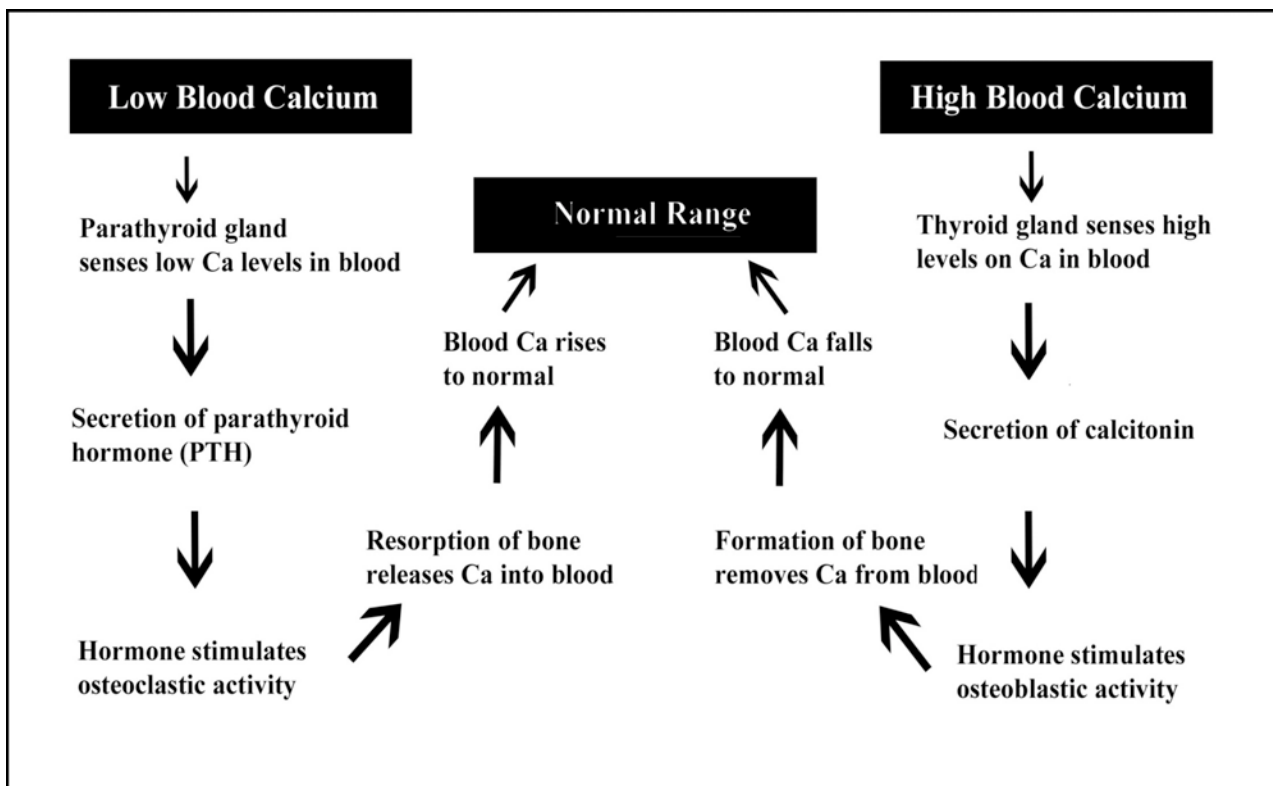


Figure 2.21: Hormonal feedback loop that controls the level of blood calcium.

Wolff's Law (Hughes-Fulford 2004; Petrtyl et al. 1996; Wolff 1892) states that bone can adapt or remodel in response to forces or demands placed upon it due to mechanical effects. Researchers disagree as to whether the primary mechanical stimulus for bone cells is stress or strain (Frost 1964; Pauwels 1965; Thompson 1917). Stress is the intensity of a distributed force (force per unit area) while strain is a measure of the degree of deformation (Nordin and Frankel 2001). The general inclination in past research is that bone responds more to strain (Bouvier and Hylander 1981; Hylander 1981; Martin and Burr 1989; Rubin and Lanyon 1982) and because of this the related variables of strain mode, direction, rate, frequency, distribution and energy must be considered in bone's adaptive abilities (Martin and Burr 1989).

For example, some past studies (Currey 1959; Evans 1957; Evans 1973) have focused on strain mode, particularly on the tensile strength of bone of different histological types. Because bone tends to be weaker under tension, the majority of failures and fractures are tension-related. Currey (1959) decided to examine how Haversian bone and non-Haversian bone reacted under tensile pressure. He classified Haversian bone as secondary, remodeled bone. Primary osteons and lamellae defined non-Haversian bone. He used oxen femora to test his hypotheses and found that more Haversian systems were present in areas of weakness. Currey (1959) believes that the Haversian systems themselves caused this weakening of bone because the actual amount of bone and the total amount of calcium are reduced when more Haversian systems are present.

Ascenzi and Bonucci (1967, 1968) also looked at the mechanisms associated with bone biomechanics. They focused on the single osteon and found evidence indicating that longitudinal systems are stronger in tension and weaker in compression than systems that pass transversely

through lamellae. However, the transversely arranged systems prove to be stronger in compression and weaker in tension (Ascenzi and Bonucci 1967, 1968).

Studies have additionally been conducted to research how habitual action (or inaction), obesity, loading, and other biomechanical factors affect the skeleton at the macroscopic level (Beaupre et al. 2000; Frost 1997; Ribot et al. 1987). Osteoarthritis, for example, causes the destruction of articular cartilage. Beaupre and colleagues (2000) state that as individuals age and subsequently decrease physical activity, their joint systems experience a reduction in loading. This alteration reactivates endochondral ossification ultimately causing a degeneration of cartilage (Beaupre et al. 2000).

Frost (1997) and Ribot and colleagues (1987) explored how obesity affects bone mass. Research indicates that obese individuals exhibit increased bone mass overall and a reduction in bone loss with age. Obesity in post-menopausal white women provided for a higher bone mineral density than for women considered to be healthy or thin (Ribot et al. 1987). Frost (1997) suggests that because overweight individuals require stronger muscle forces to move, these forces cause extra strain and stress on bone thereby resulting in an increase in bone mass and strength.

Cranial Biomechanics

For this research, it is useful to have an understanding of how the cranium reacts to biomechanical stress and trauma. The skull is comprised of twenty-two bones and thirteen sutures. It is unique due to the presence of these sutures as well as numerous foramina that serve as energy absorbers. The cranium also exhibits variations in size and shape as well as thickness

and robusticity based on sex, age, and ancestry. Head shape is vital to withstanding biomechanical forces; skulls that are more elongated can better resist compression than tension. Studies note that the degree of sutural closure can affect fracture pattern and that foramina are often the focal points of fracture origination and termination (Berryman and Symes 1998; Galloway 1999; Peterson and Dechow 2003).

As previously mentioned, bone is viscoelastic; it is stronger in compression and can withstand compressive forces better than tensile forces. It exhibits elastic and plastic characteristics and deformation. When a load is applied, it adapts due to its elastic component. When the load is removed, the bone can return to its original status. If the load is greater than the yield point, the bone moves into its plastic phase of deformation. At this point, when the load is alleviated, bone will not return to its initial state. When bone exceeds its plastic threshold, it will fail. Stress and strain increase as bone moves from exhibiting elasticity to its yield point, to plasticity, and then to failure and fracture (Currey 1970, 2002; Evans and Lebow 1951).

How and to what extent bone fractures is heavily dependent upon the rate at which a load is applied. Faster loading causes bone to fail quicker once it reaches the yield point while slower loading allows bone to go through the elastic and plastic phases and bend inward instead of failing.

Hughes-Fulford (2004) and Petrtyl and colleagues (1996) suggest that though the cranial vault undergoes biomechanical stress and strain, these factors play a more downgraded role than in long bones. Peterson and Dechow (2003) conducted an investigation into the material properties of the cranial vault (frontal, temporal, parietal, and occipital) and the zygoma. They found that in concordance with other experimental studies, bone strain in the vault are “at least

an order of magnitude smaller” (Peterson and Dechow 2003:785) than those present in long bones. They cite additional research (Endo 1966, 1970; Hylander 1987) that found that though the cranial vault does indeed bear significant biomechanical loads in specific portions, i.e., masticatory areas and nuchal musculature and attachment sites, the actual strain experienced during mastication is very low. These researchers propose that “the cranial vault is overbuilt for the function of resisting stress generated by craniofacial functions and muscular contractions” (Peterson and Dechow 2003:786). These findings may indicate that bone remodeling in the cranial vault may not be as affected by biomechanical factors as is evident in long bones.

Bone Histology and Anthropology

The Study of Histology

Bone histomorphology, or the structure of bone tissue at the microscopic level, has potential utilizations in numerous anthropological disciplines. Histological studies have led to developments in ascertaining species identification, assessing age, analyzing trauma in peri- and post-mortem environments, and understanding bone biomechanics. Histology is advantageous to the bioarchaeologist interested in disease or pathological processes in a skeletal population (Bell 1990; Ericksen et al. 1994; Martin 1991; Pfeiffer 2000). It has proven useful in paleoanthropological research to help differentiate between *H. erectus* and *H. sapiens* fossils based on quantitative measures and qualitative observations of external cranial thicknesses (Bartsiokas 2002; Martinez-Maza et al. 2006).

Such findings spurred additional progress in paleopathological research and more histological studies on skeletal material from the past. Microstructural analysis of bone can

discern pathology as well as signs of nutritional and metabolic stress (Bell 1990; Ericksen et al. 1994; Jowsey 1977; Martin and Burr 1989; Moodie 1923; Ott 1996; Pfeiffer 2000). For example, osteoporosis causes intra-cortical bone thinning and results in increased porosity within the bone. Jowsey (1977) discovered that bone depositional rates remained the same while resorption rates rapidly increased. This is caused by an extended lag time between the resorption and formation stages of ARF remodeling (Frost 1964; Martin and Burr 1989; Ott 1996; Recker 1983; Roberts and Wakley 1992). Atkinson (1965) agrees that the refilling time is prolonged in older individuals but states that increased Haversian canals and resorption spaces contribute to osteoporosis as well.

Junqueira and Carneiro (2003) describe histological indications of osteomalacia in bone. Osteomalacia is a nutritional deficiency of Vitamin D causing inadequate calcification of new bone concurrent with partial resorption of existing bone (Junqueira and Carneiro 2003). Hyperparathyroidism is a hormonal imbalance described by Robling and Stout (2000) as a condition where an excess of the parathyroid hormone is released. Though it does not have a direct affect on bone remodeling, it does affect the activation frequency (Robling and Stout 2000). They noted that individuals suffering from hyperparathyroidism have a greater number of osteons than healthy individuals.

Bone histological research (Bell 1990; Ericksen et al. 1994; Martin 1991; Paine and Brenton 2006; Pfeiffer 2000; Robling and Stout 2000) has allowed for advancements in understanding skeletal abnormalities and maladies. It has also provided new methodologies to aid the forensic anthropologist in the positive identification of human remains. The technique for mineralized tissues has been improved and honed over the past few centuries and is primarily

used today on human bone to estimate age at death and to study skeletal pathologies and nutritional stress (Ahlqvist and Damsten 1969; George 2003; Kerley and Ubelaker 1978; Moodie 1923; Stout 1989; Thompson and Gunness-Hey 1981). This study seeks to advance this aspect of the field by utilizing histomorphology to ascertain age-at-death estimations.

Age at Death and Sex Estimations

Based on what is known about this remodeling system, numerous biological and anthropological researchers have noted a correlation between age and microscopic bone characteristics and remodeling rates (Enlow 1963; Kerley and Ubelaker 1978; Kerley 1965; Stout 1989; Ubelaker 1989).

Anthropological researchers have observed that certain histomorphometric features differ significantly between males and females (Erickson 1991; Samson and Branigan 1987; Thompson 1980). Other studies have not found such strong variation between the sexes (Aiello and Molleson 1993; Clarke 1987; Kerley 1965; South and Paine 1992).

When considering the general histological structures of human bone, lamellar bone is crucial to the research. Mature lamellar bone is characterized by structural units called osteons. Osteons can be either primary (initial bone deposited on cartilage or a pre-existing surface of connective tissue) or secondary (bone formed as a result of remodeling). Secondary osteons are the focus of this study because they are the result of the remodeling process that replaces existing bone and are thus reflective of normal bone turnover as well as mechanical and metabolic stressors (Mulhern and Ubelaker 2001; Pfeiffer 2000; Ross et al.1989).

Age-at-death estimates are based on the process of bone remodeling. Because of this, the secondary osteon is commonly quantified. For example, Kerley (1965), Kerley and Ubelaker (1978), and Thompson (1979) utilized secondary osteon counts. Ahlqvist and Damsten (1969), Clarke (1987), Cool and colleagues (1995), Ericksen (1991), and Thompson (1979) examined osteon population densities. Stout and Stanley (1991) tested both secondary osteon counts and percent osteonal bone and found counts to be more accurate in predicting age estimates.

Secondary osteons are measured in several ways. Mean osteonal area, perimeter, and maximum and minimum diameters have been used to predict age-at-death estimates (Pfeiffer et al. 2006, Thompson 1979; Watanabe et al. 1998). The Haversian canal of every secondary osteon is similarly quantified utilizing average canal area, perimeter and diameters to estimate age (Thompson 1979; Thompson and Gunness-Hey 1981; Watanabe et al. 1998). Research suggests that during aging, these variable measurements decrease in response to increased bone remodeling and the addition of more secondary osteonal bone (Curtis 2003; Pfeiffer et al. 2006; Thompson 1979; Thompson and Gunness-Hey 1981; Watanabe et al. 1998).

Additionally, researchers have looked at primary osteonal counts and fractional volume of primary osteons as well as percent lamellar bone (Ahlqvist and Damsten 1969; Clarke 1987; Cool et al. 1995; Kerley 1965). As age increases, the number of primary osteons as well as the percentage of lamellar bone decrease and are replaced by secondary osteons.

Other variables considered to estimate age-at-death also include average cortical thicknesses (Curtis 2003; Thompson 1979; Thompson and Gunness-Hey 1981), secondary osteon circularity (Britz et al. 2009; Ortner 1975), and number of secondary osteonal fragments (Clarke 1987; Cool et al. 1995; Ericksen 1991; Kerley 1965).

Previous Research

Age:

Once bone is discovered in a forensic setting, researchers may utilize histological analyses to discern age-at-death to aid in the positive identification of the unknown individual (Ahlqvist and Damsten 1969; Kerley 1965; Kerley and Ubelaker 1978; Stout 1989; Stout 1992). Kerley (1965) published a unique methodological technique utilizing bone cross-sections for assessing age-at-death. He gathered quantitative data including number of whole osteons, number of fragmentary osteons, percentage circumferential lamellar bone, and number of non-Haversian canals. Kerley analyzed these variables on the anterior, posterior, medial, and lateral aspects of long bone midshafts and found a strong positive correlation between advancing age and these osteonal measurements.

Kerley's (1965) pioneering research has been highly influential in subsequent histological studies, and his microscopic estimation of age in human bone defined parameters and introduced a methodology still utilized today. Kerley (1965) noted that the outer third of the bone is least affected by resorptive and depositional changes. Others echo this finding and indicate that the endosteal border is the area of the most intense secondary bone remodeling activity (Aiello and Molleson 1993; Enlow and Brown 1958; Singh et al. 1974). The periosteal border, then, contains the structures most reflective of the actual age of the individual (Kerley 1965).

Kerley utilized a four-quadrant approach (Figure 2.22) – one circular field on the anterior, medial, lateral, and posterior periosteal regions of the midshaft femur, fibula, and tibia. He counted the number of osteons, the number of osteon fragments, and the number of non-

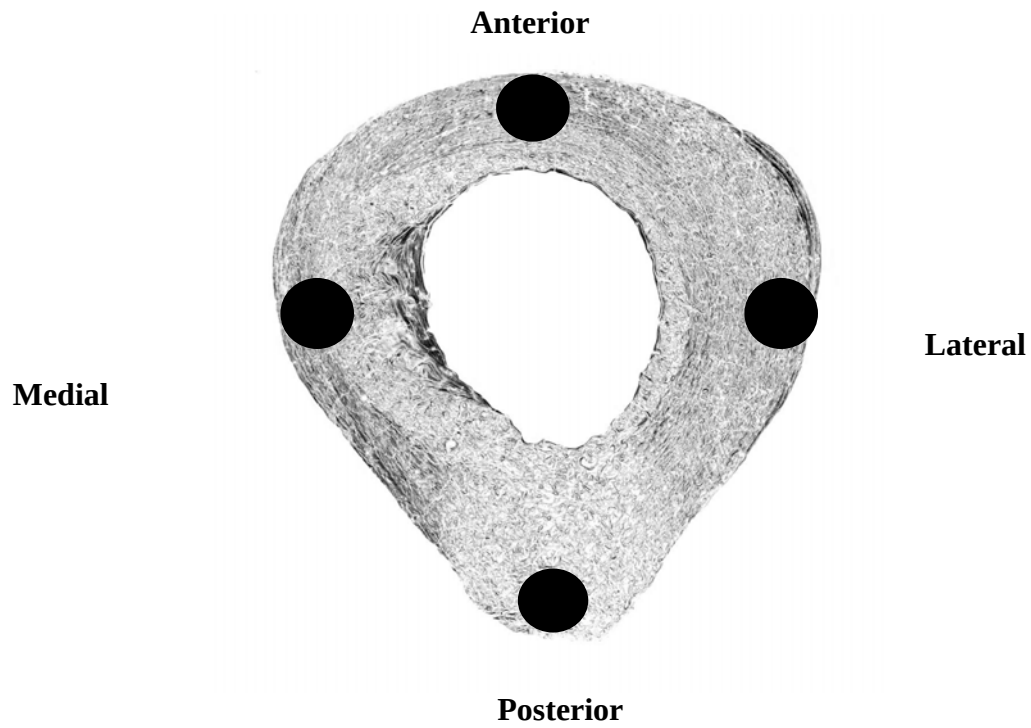


Figure 2.22: Illustration of Kerley's four quadrants in the midsection of a human femur. Black circles represent the circular fields on the anterior, medial, lateral, and posterior aspects of the bone section where Kerley gathered his data. 40x magnification.

Haversian canals. He also calculated the percentage of circumferential lamellar bone. He constructed age graphs and regression equations for each variable to predict age based on histological variables. He used “normal” specimens only for the regression equations but noted and hypothesized (though did not test) that pathology and nutritional and metabolic deficiencies would most likely be incorrectly aged due to changes in microstructure. Kerley found that the osteonal fragments in the outer 1/3 of the fibula presented the most accurate method to estimate age-at-death in human remains. The Kerley method has been modified and retested; variations have been introduced and new methods and suggestions based on his initial research have been published.

Ahlqvist and Damsten (1969) presented one of the first modifications based on the following problems with Kerley’s techniques: 1) difficulty distinguishing between and defining osteons from osteon fragments, 2) the rough estimation of the percentage of circumferential lamellar bone in a circular visual field, and 3) the difficulty in deciding the kind of structure visible on the outer edges of the circular field. They (1969) suggested a few changes. Still utilizing Kerley’s “four-field” approach, they used squares instead of circles (Figure 2.23). The use of a squared ocular field was proposed to alleviate the difficulty of estimating lamellar bone percentage and recognizing osteons versus osteon fragments. Ahlqvist and Damsten (1969) also moved the fields from the most anterior, medial, lateral, and posterior to locations that fell between Kerley’s areas of interest. They found it difficult to interpret Kerley’s (1965) definitions of complete versus fragmentary osteons, so instead of counts, they used percent osteonal bone to estimate age from the femora midshaft. Their results proved to be less accurate overall than those produced by Kerley (Ahlqvist and Damsten 1969; Stout 1992).

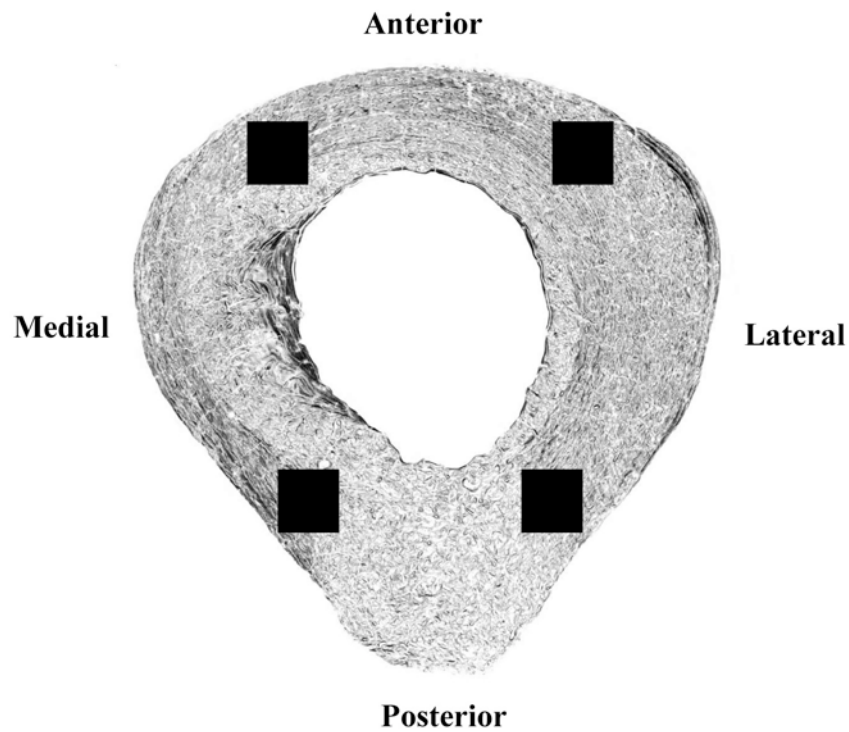


Figure 2.23: Illustration of Ahlqvist and Damsten's (1969) modification to Kerley. The squares represent aspects of the femoral mid-section where data were gathered. 40x magnification.

Following this modification, Kerley and Ubelaker (1978) decided to re-test the original Kerley methodology against new Ahlqvist and Damsten (1969) research. They found errors in the initial publication noting that the original field size was erroneous as were some of the regression equations. Kerley and Ubelaker (1978) noted the correct field size of 1.62 mm rather than 1.25mm and tweaked the regression equations.

Other researchers have also proposed modifications to Kerley's original methodology. One of the most prolific concerns about his method is the use of an entire cross-section of bone. This is destructive since it sections the bone at midshaft. Thompson (1979) introduced a core technique method to determine age-at-death (Figure 2.24). Instead of an entire cross-section of bone, a 0.4cm diameter core was removed from different areas of the femur, tibia, ulna, and humerus midshaft. Thompson utilized almost 20 variables and formulated regression equations. However, his estimates were not as significantly correlated with age as were Kerley's (Stout 1992; Thompson 1979). Ericksen (1991) also suggested a modification to the Kerley technique. Instead of complete cross-sections, a wedge of bone should be removed from the femoral midshaft. Her results also failed to produce the reliable equations presented by Kerley (Ericksen 1991).

Aiello and Molleson (1993) looked at the differences in the accuracy of using macroscopic versus microscopic means to estimate age-at-death of individuals from Christ Church Spitalfields remains. They cited Kerley's (1965) method as the most reliable histological technique and thus tested its reliability, with some modifications, on their sample of femoral cross-sections. They utilized Kerley's (1965) circular fields but Ahlqvist and Damsten's (1969)

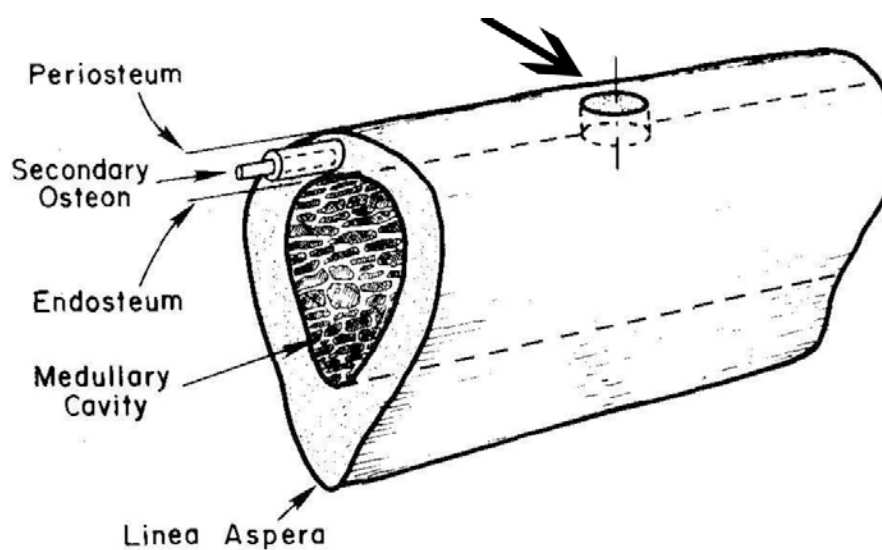


Figure 2.24: Thompson's core technique illustrated on the femur. The area removed for analysis is indicated by the arrow. Adapted from: Thompson, 1980.

field position. They also redefined secondary osteons and fragments so that an osteon was whole if the central canal was not affected by the remodeling process.

Aiello and Molleson (1993) also found a significant correlation between age and the variables though noted that the Spitalfields sample retained bone for longer, or remodeled at a slower rate, than did the sample on which Kerley developed his regression formulae. The ages produced were still “reasonable” (Aiello and Molleson 1993). Samson and Branigan (1987) moved away from the outer 1/3 of the cortex to the middle to quantify secondary osteons in a cross-section. Their study resulted in considerably lower correlations between known age and the structures than those of Kerley (Aiello and Molleson 1993). Results indicate that histological features in the middle of the cortex do not reflect age as well as they might at the periphery of the cortex. So, the Kerley technique still prevails (Aiello and Molleson 1993).

An additional worthy study of mention is that of Stout and Stanley (1991). These researchers tested Kerley’s (1965) osteon count method versus Ahlqvist and Damsten’s (1969) percent osteonal bone method. Percent osteonal bone would be more useful than osteon counts when remains are fragmentary or when bone is less well-preserved. They also looked for a stronger correlation between variables and age when using the four-field approach or when observing the entire cross-section. Stout and Stanley (1991) found that more accurate age-at-death estimations result from Kerley’s (1965) osteon count method. They found that utilizing Kerley’s count method in the entire cross-section of the fibula produces the best results.

Kerley’s research has indeed been a stronghold in subsequent studies in the 1970s, 80s, and 90s. Numerous facets have been questioned and retested, but the method has maintained the most reliable age-at-death estimations. Problems still exist with the Kerley methodology. To

achieve accurate age estimations, his technique must be perfectly followed (Stout 1992). This is difficult when fragmentary remains in a forensic context are found. Having the entire and intact midshaft section of a long bone is not always feasible or probable. Tersigni (2005) questioned whether the distribution of structures varied from the midshaft to the distal and proximal ends. Kerley (1965) stated that there is a block in the midshaft of the bone about three inches in length that is homogenous in microstructure. However, what happens when his method is applied to the ends of the bone? Tersigni (2005) discerned that age estimations are not as reliable or accurate when applying the Kerley technique to more distal or proximal sections of long bones.

Despite the questions and drawbacks, however, Kerley's method will continue to be the foundation upon which future research is based. His initial definitions and methodology have given recognition to the value of utilizing structures to estimate age-at-death in human bone and his work will continue to be a stimulus for additional questions, revisions and techniques.

Sex:

Kerley's (1965) age-at-death research stated that sex differences did not affect the microscopic age changes quantified in his method. However, Kerley's (1965) sample included eighty-eight males, twenty-nine females and nine individuals of unknown sex. Such an uneven distribution may have affected this conclusion.

Stout and Paine (1992) tested histological methods for estimating age at death by using osteon population densities for the rib and clavicle. They combine both male and female individuals and do not suggest the need for sex-specific equations. Like Kerley (1965), Stout and Paine (1992) had a skewed sample distribution with thirty-two males, seven females and one individual of unknown sex.

Other researchers have noticed significant differences between male and female histomorphometrics (Ericksen 1991; Samson and Branigan 1987; Thompson 1980). Ericksen (1991) removed small bone wedges from anterior midshaft femora and considered counts of secondary osteons, secondary osteon fragments and non-Haversian canals as well as average percentages of un-remodeled bone, osteonal bone and fragmental bone. She developed linear regression equations separately for males and females as well as for combined sexes and found that the number of secondary osteons and secondary osteon fragments do differ between males and females and, because of these findings, recommends the importance of using sex-specific analyses (Ericksen 1991).

Samson and Branigan (1987) also utilized the femur and procured samples from the anterior-medial and anterior-lateral aspects of midshaft cross-sections collecting mean cortical thickness, mean minimum Haversian canal diameter, and number of Haversian canals per unit area of bone. They analyzed males and females separately and were only able to find significant correlations between age and histomorphometrics for male individuals. Their research did not find a similar strong relationship for females and they suggest this is due differences in bone remodeling between the sexes (Samson and Branigan 1987).

Thompson (1980) states that long bones lose cortical thickness and become more porous as individuals get older. The goal of his research was to quantify such changes and considered bone mass and density as well as Haversian canal number and size. His findings indicate that females experience a much more significant decline in cortical thickness and bone mineral mass with increasing age than do males. Thompson (1980) also noted significant age-related changes in Haversian canal areas with females having greater values than males.

The above findings suggest that males and females undergo bone remodeling at different rates. This lends credit to the potential utility of histomorphometrics to estimate sex.

Limitations

There are numerous limitations that restrict the applicability of the above age estimating methodologies. Because of these issues with age estimation techniques, the noted differences found in the above-mentioned literature between male and female histomorphometrics may not be accurate either.

Many limitations restrict the applications of Kerley's (1965) method:

1) wholesale utilization as he used only the femur, tibia, and fibula; 2) only the midshaft and 3) all measurements on transverse sections. This is problematic when there are no limbs or bones are fragmentary. The midshaft may not always be available or a transverse section not possible. Tersigni (2005) also suggests that if an age estimate is made on areas other than the midshaft there is a higher probability that that estimate will be significantly different than that at midshaft. Therefore, application of the Kerley method may not always be accurate.

Another limitation of using the post-cranial skeleton is biomechanical stress and strain (Ascenzi and Bonucci 1967, 1968; Carter and Beaupre 2001; Currey 1959, 1970). Bone grows or models based on its ultimate structure and function. Cortical drift can occur during this process and differentially affect osteon density in a single transverse section of a long bone as well as in multiple areas throughout the shaft.

Bone remodels in response to forces, demands and strain placed upon it. However, it often does so in a non-uniform manner because any load or deformation it experiences is not necessarily of equal magnitude in all parts of the bone. Bone resorption and formation follow a

specific sequence but the two processes may not always be working in the same place on the bone (Bouvier and Hylander 1981; Frost 1964; Lanyon and Bourn 1979). Estimating age-at-death from these areas would affect the accuracy of histological aging techniques. For example, in an anthropological study, Kerley (1965) found a greater variation in number of osteons and osteon fragments not correlated to age in the posterior field. Ahlqvist and Damsten (1969) suggest that this finding results from the posterior circular field falling on the femoral crest – an area of powerful muscle insertions.

The neurocranium does have muscle attachment sites, but stress and strain are hypothesized to affect these areas less (Laval Jeantet et al. 1983) so the presence of increased differential remodeling due to loading should decrease in the vault bones thus offering a more accurate age-at-death estimation. Since such intra-bone variation has been demonstrated to exist in the post-crania, thus affecting age estimating regression equations, this research utilizes neurocranial bone to test whether a similar amount of variation exists or whether the vault bones actually provide a more reliable age-at-death estimation.

Neurocranium for Age and Sex Estimations

Cool and coworkers (1995) and Clarke (1987) were first to investigate the utility of using the histological traits of vault bones to ascertain age-at-death. Clarke (1987) considered the parietal and Cool et al. (1995) examined the occipital. Curtis (2003) examined the microscopic appearance of the frontal bone in relation to age. Clarke (1987) and Curtis (2003) addressed the sex distribution of their samples and whether or not sex-pooled or sex-specific analyses should be conducted.

Clarke (1987) attempted to develop regression equations to estimate age-at-death using histological features of the parietal bone including fractional volume of secondary osteons, secondary osteon fragments, and un-remodeled lamellar bone. He extracted three bone cores from the right aspect of the parietal (Figure 2.25) for analysis. The three cores were situated midway between the parietal eminence and bregma, lambda and the sagittal suture. He noted extensive variation but was unable to reach conclusive results because of such variation.

Clarke (1987) did not find significant differences in the microstructure between males and females. However, his research was skewed due to a small sample size ($n=36$) and biased towards males over the age of seventy. There were twenty-three males, twenty over the age of seventy, and thirteen females, nine over the age of seventy. All of the individuals in the sample were older than fifty (Clarke 1987).

Cool and colleagues (1995) examined the value of the microscopic traits of the occipital bone in developing regression equations. They removed bone cores from the left side of the occipital bone in males only at a standardized distance from lambda (Figure 2.26). Cool et al. (1995) considered the same measurements as Clarke and added in the variable of fractional volume of primary osteons. They examined three random fields in both the endocranial and ectocranial tables. Because of the irregular topography of this cranial bone, osteonal organization was non-uniform and the research was also inconclusive.

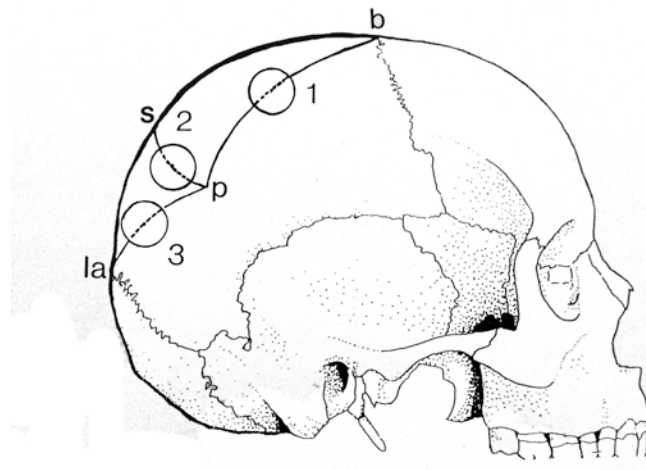


Figure 2.25: View of skull depicting core-sampling locations (sites 1, 2 and 3) for Clarke's 1987 research. Each core was removed at an area midway between the parietal eminence and bregma, lambda, and the sagittal suture. (b-bregma, l-lambda, s-sagittal suture, p-parietal eminence).

Adapted from: Clarke (1987)

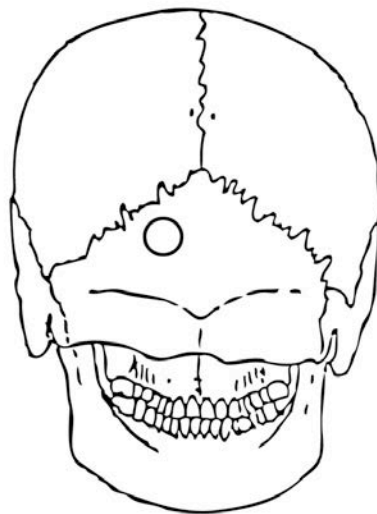


Figure 2.26: Approximated area on the left aspect of the occipital where Cool et al. (1995) procured bone core samples for analysis.

Curtis's (2003) study addresses the utility of frontal bone histomorphometrics. She notes that in previous neurocranial studies (Clarke 1987; Cool et al. 1995), secondary osteon size was not measured or controlled for. In her research, Curtis took bone cores from the left and right aspects of the frontal eminence (Figure 2.27) of ninety-two modern cadavers described by the author to be of European American ancestry.

She considered fourteen different variables and looked at whether or not the measurements differed depending on the orientation of the bone core (perpendicular, parallel, or at a forty-five degree angle to the sagittal suture) (Figure 2.28) or on the area sampled (left or right side of the frontal bone). She also addressed whether these variables were significantly affected by sex. Curtis learned that orientation did not affect the variation of measurements in the frontal bone. Yet, she did find that side affected certain measurements, i.e., osteon size, but was irrelevant in reference to others, i.e., Haversian canal size.

Curtis (2003) demonstrated that sex was a significant factor in measurements of external table thicknesses and osteon density. Microscopic features of the frontal bone did correlate with increasing age. This is an important advancement in research and though Curtis notes that the frontal bone is less affected by age than other skeletal elements, the utility of it as a means to estimate age-at-death is promising. It is important to note, however, that her large sample size is primarily comprised of individuals over the age of sixty. It is necessary to continue neurocranial histomorphometrics by expanding the younger to middle age samples.

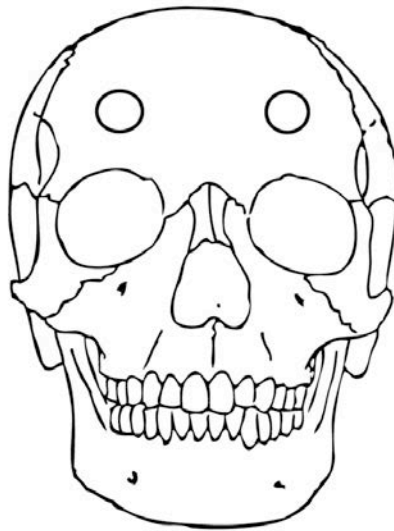


Figure 2.27: Approximated locations of bone cores removed by Curtis (2003) from the left and right frontal eminences.

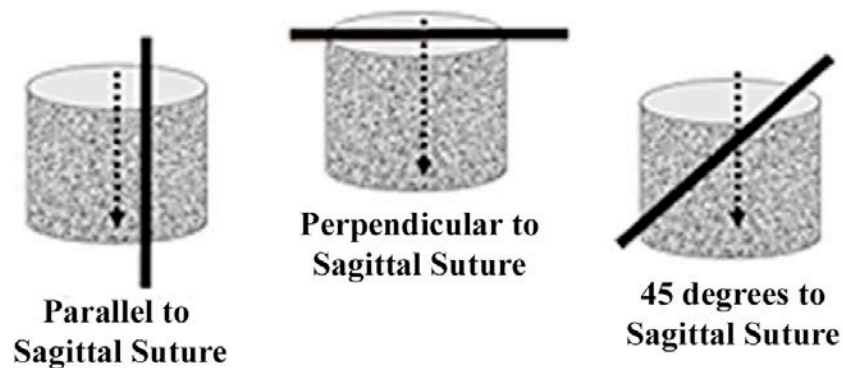


Figure 2.28: Bone cores were sectioned parallel to, perpendicular to, or at a 45-degree angle to the sagittal suture to test for differences in measurements based on orientation. The dotted line represents the sagittal suture. The solid lines represent the direction the bone was sectioned in relation to the sagittal suture.

Adapted from Curtis (2003).

Historically, in anthropology, the majority of histological analyses have focused on postcranial bones and specifically, the pelvic and pectoral limbs. Few studies have undertaken a characterization of flat vault bones and it is not uncommon to discover a skull or fragmented neurocranial elements.

A pilot study (Trammell et al. 2008) at the Regional Forensic Center at the University of Tennessee Medical Center revealed that the structures commonly found in long bones are also visible in the neurocranium. Human cranial samples of the frontal, parietal and temporal were secured at autopsy from individuals of known age, sex and ancestry. These bone samples were cleaned, embedded and sectioned at a thickness of approximately thirty microns. Microscopic evaluation of these slides demonstrated that the necessary histomorphological features utilized in the development of age-at-death regression equations in long bones are also present in neurocranial elements. It is quite possible, then, to utilize the vault bones to estimate age-at-death.

The current research will focus on the histomorphometrics of the frontal, parietal and temporal to test their utility in assessing sex and estimating age. The age distribution will concentrate on individuals under the age of fifty years to address the absence of these younger groups in previous research (Clarke 1987; Cool et al. 1995; Curtis 2003).

Chapter 3: Materials and Methods

This research was conducted at the Mineralized Tissue Histology Laboratory at the University of Tennessee, Department of Anthropology in Knoxville, Tennessee, the Forensic Anthropology Laboratory at the Regional Forensic Center (RFC), University of Tennessee Medical Center in Knoxville, Tennessee, the Pima County Office of the Medical Examiner in Tucson, Arizona, and the University of Arizona College of Medicine in Tucson, Arizona. The first laboratory provided access to equipment and supplies for embedding samples. The additional three facilities allowed for thin section preparation, i.e., cutting, grinding and polishing, microscopic evaluation, and data collection and documentation.

Samples

Neurocranial samples of human frontal, parietal, and temporal bone were secured at autopsy from forty-six individuals of known age, sex and ancestry from the RFC at the University of Tennessee Medical Center in Knoxville, Tennessee. Additional samples were procured from two modern forensic cases donated to the RFC. Based on Curtis' (2003) findings that histomorphometrics varied based on side, these square samples were all removed from the left side of the skull and measured approximately 1x1 centimeters (cm) (Figure 3.1). They were taken from white males and females of a wide age distribution. Crania with obvious pathologies or visible trauma were excluded. Complete medical histories are available for each specimen, so retrospectively, if outliers exist due to differential bone remodeling or atrophy, such causes can potentially be traced to underlying disease or deficiencies.



Figure 3.1: Examples of neurocranial samples prior to embedding (cm scale).

Care was taken to procure individuals from each decade ranging from the late teens and twenties up to seventy years of age; however, due to the demographics of those autopsied, this study leans more towards individuals under the age of sixty. The age distributions for this research are illustrated in Table 3.1. Figure 3.2 compares the age of the samples in this study to sample distributions from previous cranial histological endeavors.

Sample Procurement

Samples were procured from forty-six autopsied individuals and two modern-day skeleton donations at the Regional Forensic Center. One-by-one centimeter squares were sectioned from the same region of the left aspect of the frontal, parietal, and temporal bone for all individuals using a *Stryker*® (Stryker Corporation, Kalamazoo, Michigan) saw (Figure 3.3). Specimens were procured in areas void of cranial landmarks, i.e. glabella, buttressing, or sutures. After sample procurement was completed, the individuals over the age of fifty years (n=9) were removed from the study. This allows for a more even distribution between present age groups and prevents bias in the results due to so few older individuals.

Table 3.1 Age and sex distribution of sample collection

Age Range	Total N	Males	Females
20-29	13	8	5
30-39	12	6	6
40-49	14	8	6
50-59	6	2	4
60-69	2	1	1
70-79	1	1	0
Total	48	26	22

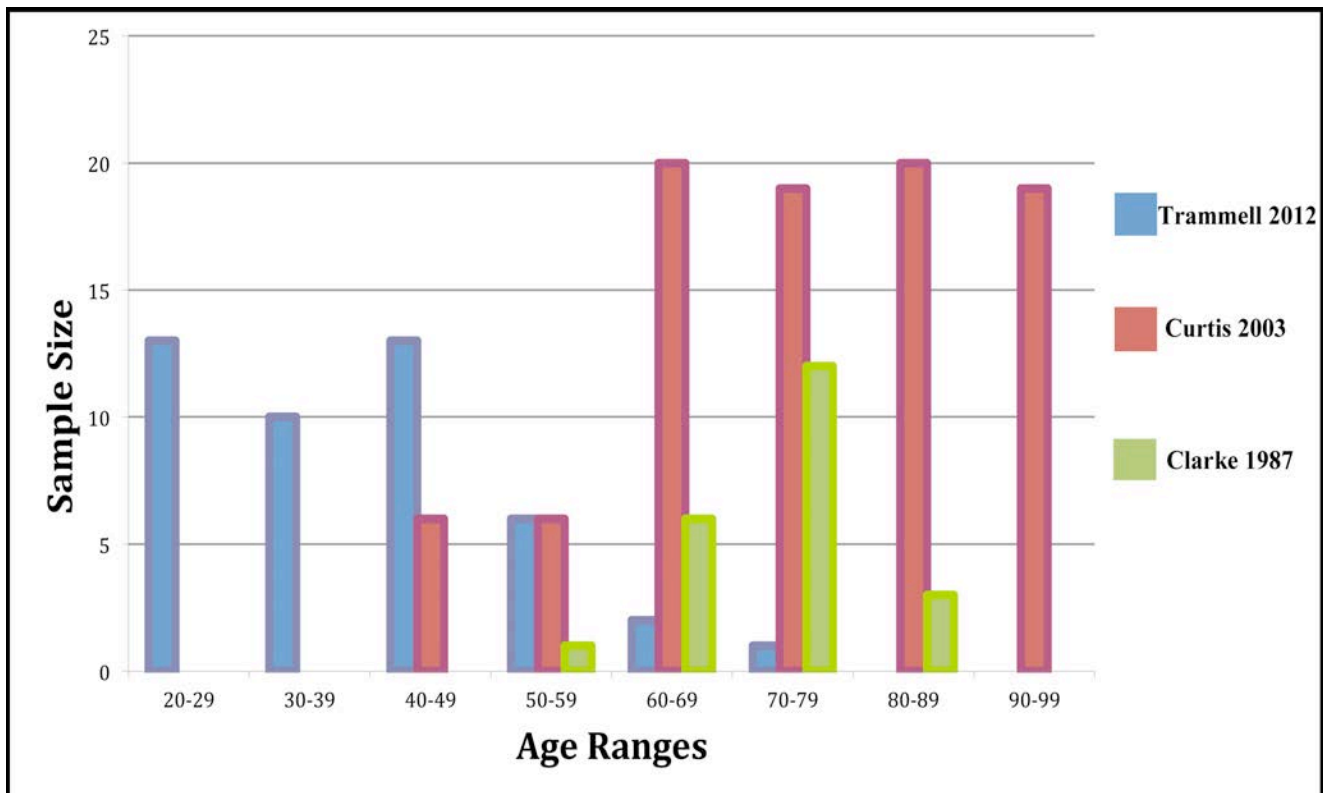
**Figure 3.2: Age distribution of this research compared to previous research. Notice how the current study focuses on individuals under the age of fifty.**



Figure 3.3: Lateral view of a skull showing the sections made at autopsy and approximate location of the sampled regions of the frontal, parietal and temporal (cm scale).

Once removed, the samples were placed in vials of formalin and numerically marked with the case number and bone. After the samples were obtained, they were transported to the University of Tennessee Department of Anthropology Mineralized Histology Laboratory to be similarly cleaned with distilled water to remove any adhering periosteal tissue. The samples were then laid out and allowed to dry for 48 hours prior to embedding.

Thin-Section Preparation

When samples were cleaned and completely dried, they were embedded in solution of a mixture of five parts *Buehler® EPO-THIN®* (Buehler, Lake Bluff, Illinois) resin and two parts *Buehler® EPO-THIN® hardener* (Figure 3.4). Two thin-sections were prepared from each block using a *Buehler® Isomet® Low Speed Oil-cooled Diamond Saw* (Buehler, Lake Bluff, Illinois) (Figure 3.5) to ensure that at least one section would be suitable for analysis. Samples were ground using 600 grit papers and polished using a *Dual Wheel Mark V® Laboratory* (Mark V Laboratory, East Granby, Connecticut) grinder/polisher (Figure 3.6) prior to examination of histomorphological features.

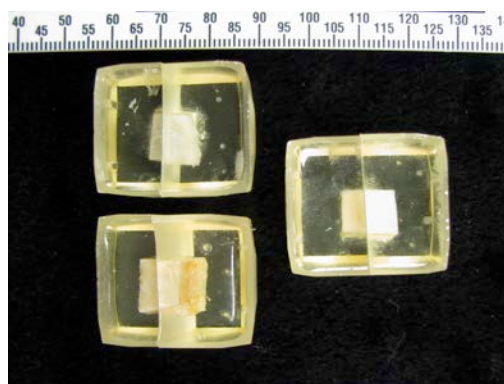


Figure 3.4: Example of three embedded neurocranial samples prior to thin-section (mm scale).

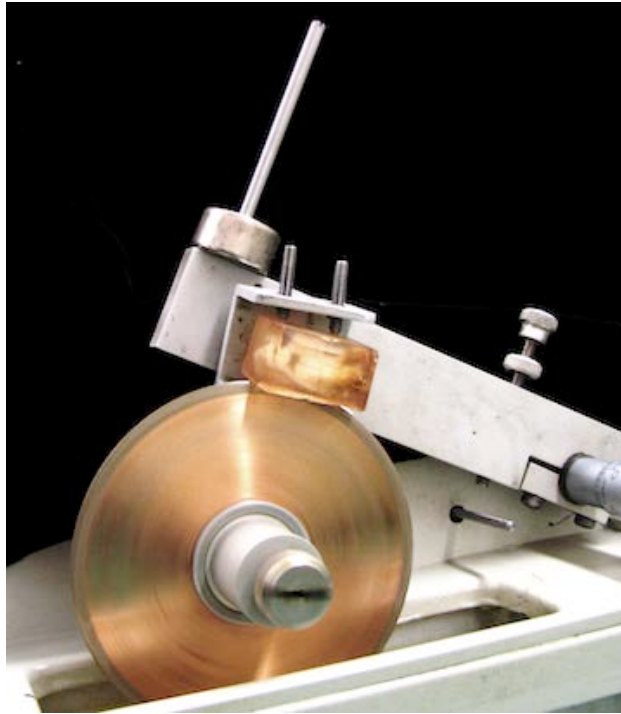


Figure 3.5: *Buehler® Isomet® Low Speed Saw* with sample block being sectioned with a diamond wafering blade.



Figure 3.6: *Dual Wheel Mark V® Lab Grinder and Polisher.*

Each sample was given a numeric. These data and the corresponding ages and sex were recorded in a Microsoft excel datasheet as well as in a laboratory notebook. The catalog numbers were etched onto each petrographic slide for identification purposes.

Microscopic Examination

The histological analysis utilizes a combination of protocols from Curtis (2003), Kerley (1965), and Kerley and Ubelaker (1973). Kerley (1965) examined transverse sections from femur, tibia and fibula midshafts and quantified the number of osteons, fragments of old osteons, percent of circumferential lamellar bone (primary bone) and number of non-Haversian canals. He did so within each section at four fields situated at the anterior, posterior, medial and lateral portions of the section. He examined a circular area of 2.16mm^2 in each field. In this study, because of the smaller sample size, measurements were taken over the entirety of both the inner and the outer cortices of each section as opposed to a four-field approach – similar to Curtis (2003).

These sections were viewed and digital images were captured via a *Leica® DMRX* light microscope (Leica Microsystems, Wetzlar, Germany) at 1.6x/0.05, 40x, 100x, and 200x magnification and an accompanying *Sony® DXC-5500* (Sony Electronics Inc. San Diego, California) digital camera (Figure 3.7). Measurements were taken from 1.6x/0.05 magnification and 100x magnification captured images via *Image-Pro® Express* (Media Cybernetics, Inc., Bethesda, Maryland). All histological images depicted in this chapter and in subsequent chapters are micrographs. The digital images of each slide as well as measurement data are available and accessible at the Forensic Anthropology Laboratory at the Regional Forensic Center, University of Tennessee Medical Center in Knoxville, Tennessee.



Figure 3.7: Leica® DMRX light microscope with attached digital camera.

The overall area of each slide was first examined to recognize cellular structures, evidence of remodeling and the distribution of remodeling prior to quantification of the following features: external table thickness, secondary osteon area, secondary osteon perimeter, secondary osteon maximum and minimum diameters (and ratios), secondary osteon Haversian canal area, secondary osteon Haversian canal perimeter, and secondary osteon Haversian canal maximum and minimum diameters (and ratios).

Image-Pro® Express can mark and number each measurement; this benefit ensured that each osteon and its features were measured and counted only once. Each feature is referred to in the proceeding text as a sub-variable. This term was utilized in similar research (Curtis 2003) and has thus been adapted for the purposes of the current research.

Measurements

Measurements utilize the same definitions of sub-variables as described by Curtis (2003), Kerley (1965), Kerley and Ubelaker (1978), and Keough (2007) (Table 3.2). The statistical analysis utilized eleven of these. The twelfth sub-variable, total osteon counts, was not included because of the amount of variation in the sample area (length x width) of each bone. External table thicknesses were not collected from the temporal bone samples due to the difficulty in differentiating between internal and external tables and the diploe.

Table 3.2 Histomorphological variables

Number	Sub-variable	Abbreviation
1	External table thickness	ExtTable
2	Total number of secondary osteons	Total
3	Secondary osteon area	OA (nm ²)
4	Secondary osteon perimeter	OP (nm)
5	Secondary osteon max diameter	OA max (nm)
6	Secondary osteon min diameter	OA min (nm)
7	Haversian canal area	HCA (nm ²)
8	Haversian canal perimeter	HCP (nm)
9	Haversian canal max diameter	HC max (nm)
10	Haversian canal min diameter	HC min (nm)
11	Secondary osteon diameter ratio	OD ratio (nm)
12	Haversian canal diameter ratio	HC ratio (nm)

* nm = nanometers

External Table Thickness (ExtTable): Significant differences in external cortical thickness were found between males and females according to Curtis (2003). This implication as well as the relationship between thickness and age was examined. To estimate external table thickness, fifteen cortical thickness measurements were taken perpendicular to the external table using the trace tool in *Image-Pro® Express* (Figure 3.8). These measurements were then averaged. The presence of adipocyte-filled lumen (Figure 3.9) was noted on some of the sections. These extend from the diploe into the external table but were never uniform in appearance and distribution. These lumen may affect the accuracy of the external table thickness measurements so such areas were avoided if possible. Neither the diploe nor any areas of abnormality or pathology were included in these thickness calculations.



Figure 3.8: Example of how measurements of cortical thicknesses were taken on external table of the frontal bone. 1.6x/0.05 magnification.

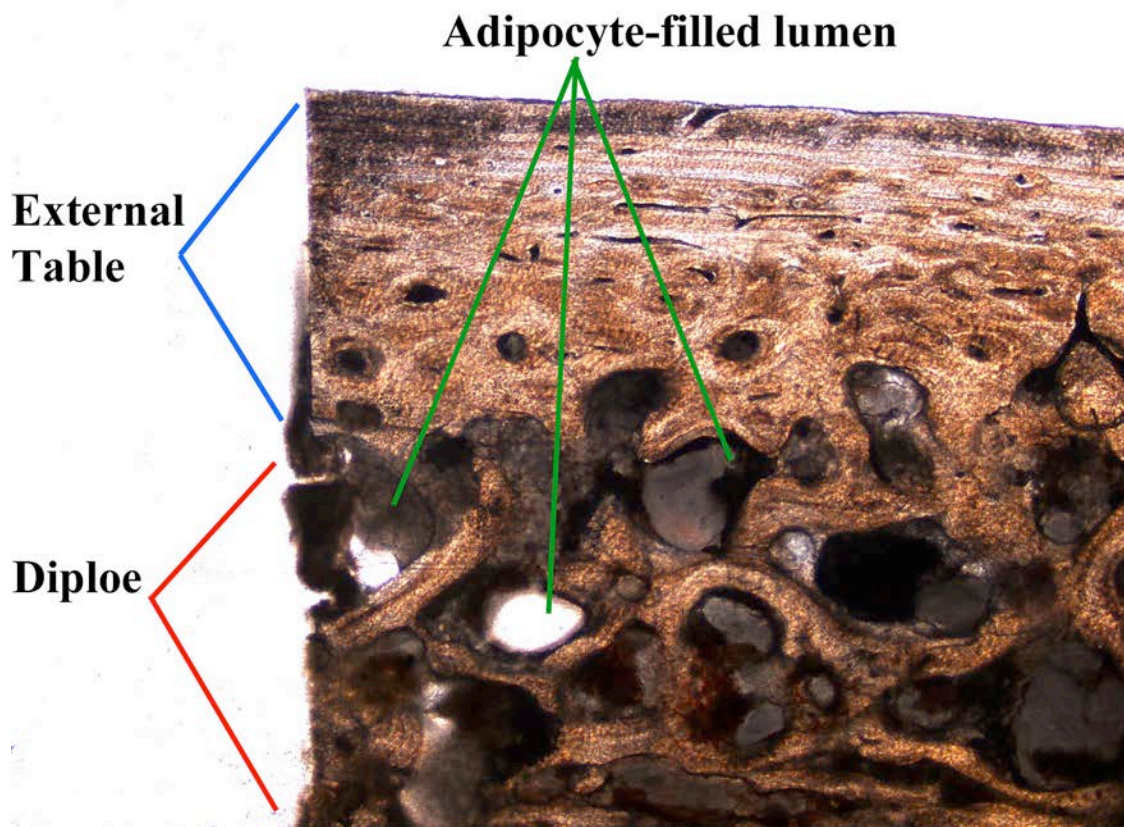


Figure 3.9: Prepared thin-section of the external table of the frontal bone. There are adipocyte-filled lumen extending from the diploe into the external table. 1.6x/0.05 magnification.

Total number of secondary osteons (Total): An osteon or Haversian system can be seen in cross-section as a vascular canal surrounded by concentric lamellae. A reversal line delineates the periphery of the osteon and marks the area where osteoclastic resorption stopped and was followed by new bone formation (Figure 3.10). Unless otherwise specified, osteon refers to secondary bone. The total number of complete osteons is counted and area measurements were recorded for each of these osteons and corresponding Haversian canal (Figure 3.11). For the purposes of this research, a secondary osteon is considered complete as opposed to fragmentary (3.12) when the entirety of the Haversian canal is circular and fully observable and the cement line is visible for the whole osteon.

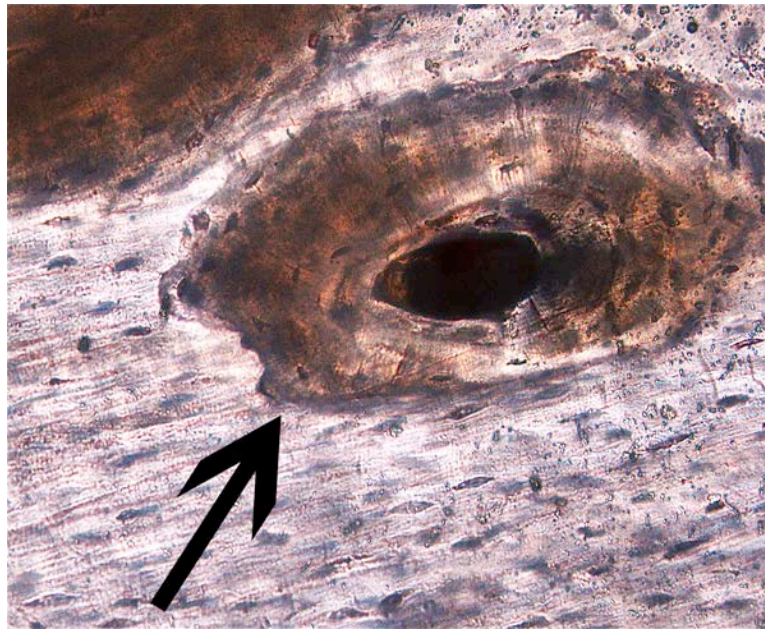


Figure 3.10: Arrow indicates a complete secondary osteon in external table of the frontal bone surrounded by reversal line. 200x magnification.

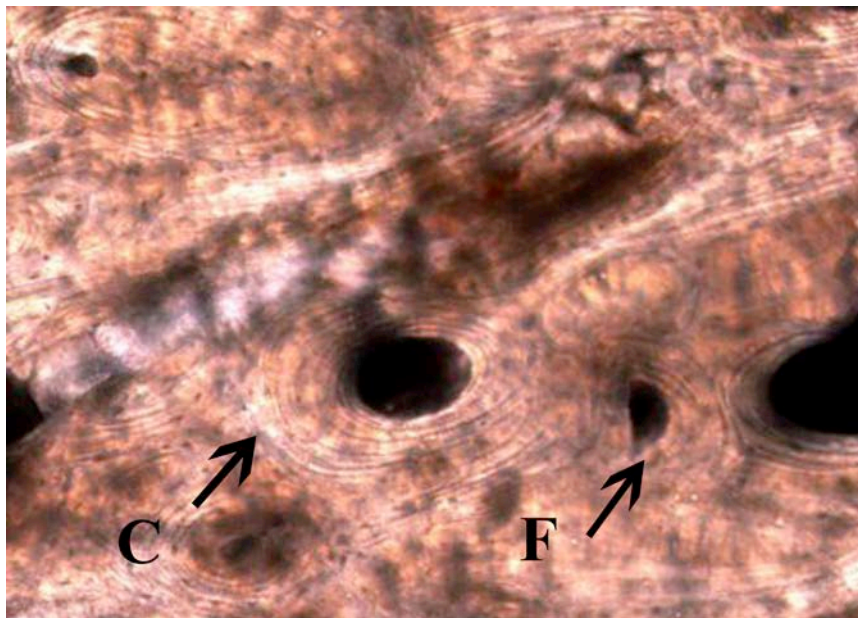


Figure 3.11: Complete and fragmentary secondary osteons in the parietal bone. The complete osteon (C) is visible in its entirety while the fragmentary osteon (F) is obscured by the introduction of new secondary bone via remodeling. 200x magnification.

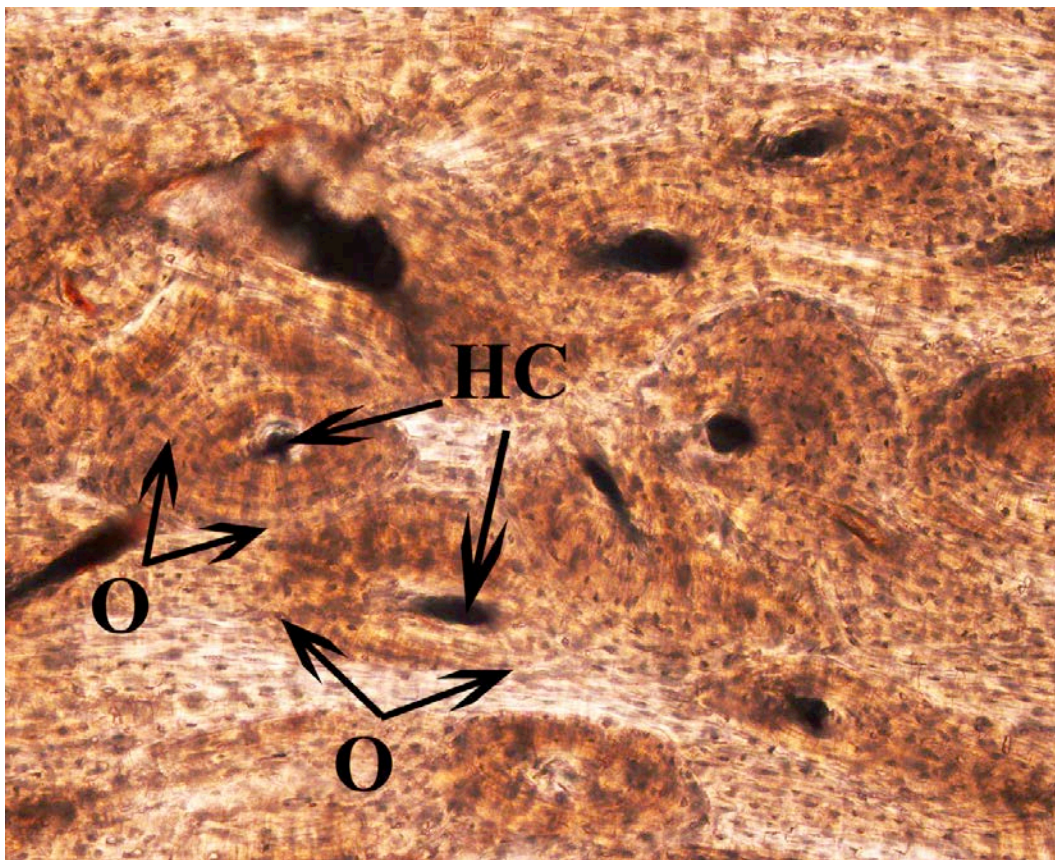


Figure 3.12: Arrows indicate some of the secondary osteons (O) and Haversian canals (HC) in this thin-section of the frontal bone. 100x magnification.

Secondary osteon area (OA) and perimeter (OP): For every complete secondary osteon, the squared area (nm^2) and perimeter (nm) of the osteon itself were measured. The area trace tool in *Image-Pro® Express* outlined the osteonal reversal line to acquire these metrics (Figure 3.13)

Haversian canal area (HCA) and perimeter (HCP): For every complete secondary osteon, the corresponding Haversian canal area (nm^2) and perimeter (nm) were measured via the area trace tool in *Image-Pro® Express* (Figure 3.14).

Secondary osteon maximum and minimum diameters (OA max, OA min): For every secondary osteon included in the total number count, maximum and minimum diameter measurements (nm) were recorded for the osteon using the trace tool in *Image-Pro® Express* (Figure 3.15).

Haversian canal maximum and minimum diameters (HC max, HC min): For every secondary osteon included in the total number count, maximum and minimum diameter measurements (nm) were recorded for the corresponding Haversian canals using the trace tool in *Image-Pro® Express* (Figure 3.16).



Figure 3.13: Complete secondary osteon outlined in black via trace tool in *Image-Pro® Express*. This tool provided area and perimeter measurements. 100x magnification.



Figure 3.14: Haversian canal outlined in white via trace tool in *Image-Pro® Express*. This tool provided area and perimeter measurements. 100x magnification.

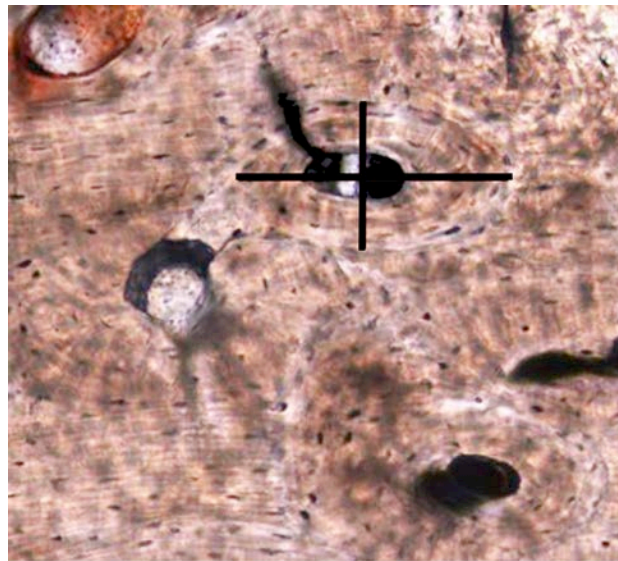


Figure 3.15: Maximum and minimum diameters of complete secondary osteon noted by black lines. The trace tool in *Image-Pro® Express* took these length metrics. 100x magnification.

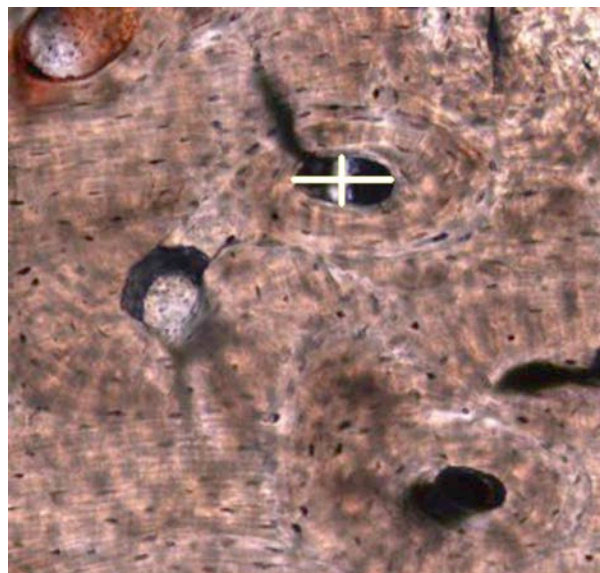


Figure 3.16: Maximum and minimum diameters of Haversian canal noted by white lines. The trace tool in *Image-Pro® Express* took these length metrics. 100x magnification.

Statistical Analysis

Overall, 142 slides were analyzed and their twelve sub-variables quantified. Statistical software programs *JMP 9.0* and *SAS 9.2* were utilized for these analyses. Four null hypotheses were first considered prior to creating regression equations to estimate age-at-death. These hypotheses are as follows:

- H₀1: There are no significant differences in the mean values for each sub-variable in the internal and external tables.
- H₀2: There are no significant differences in the mean values for each sub-variable between males and females.
- H₀3: There are no significant differences in the mean values for each sub-variable between bone by sex.
- H₀4: The observations are independent (basic assumption of regression analyses)

After data was gathered for the first twenty-four samples, a Repeated Measures ANOVA compared the mean values for each sub-variable by table location (internal table versus external table). These sub-variables included: secondary osteon area, secondary osteon perimeter, secondary osteon maximum and minimum diameters and ratios, secondary osteon Haversian canal area, secondary osteon Haversian canal perimeter, and secondary osteon Haversian canal maximum and minimum diameters and ratios. The ANOVA was used because the data matrix was not set up as a paired samples matrix.

The ANOVA assumption of normality was assessed using the Shapiro-Wilks test and the assumption of equal variance was tested using the Levene's F test for each sub-variable. Both assumptions were met for all sub-variables.

Since there are six ANOVAs, the significance value for rejecting the null hypothesis should be adjusted for multiple testing. Therefore, for the stipulated criterion of $\alpha = 0.05$, a Bonferroni adjusted alpha would be: $0.05 / 6 = 0.008$. Since there were no significant

differences between the internal table and external table means for any of the sub-variables, the remainder of the data collection process focused only on the external table of each thin-section.

To determine if significant differences exist between the means of the sub-variables by sex for each bone, a 2-tailed T-test was conducted. Since $\alpha=0.05$, the two-tailed t-test allots 0.025 of the statistical significance towards each “tail” because results in either direction will be useful for additional analyses. If the reported p-value is less than alpha, then the hypothesis is rejected and the means across the sexes are not equal. Because results of this test indicate there are some significant differences between males and females, they were analyzed both together and separately for the remaining analyses.

Next, the differences in the means of the sub-variables between bones (for males and females separately) was determined using the Tukey-Kramer Honestly Significantly Different (HSD) post-hoc test. The criterion of $\alpha=0.05$ was used in this analysis to test for statistically significant differences in the data. If the reported p-value is less than alpha, then the hypothesis is rejected and the means across bones are not equal. Since results indicate the necessity of focusing on each individual bone, the frontal, parietal and temporal were considered separately for subsequent statistical analyses.

One of the primary assumptions of regression analyses is the independence of observations (Kirkwood 1988; Madrigal 1998). Multiple observations taken from one single sample are likely correlated. The major problem with using correlated measurements in regression is that the calculated p-values are not correct, resulting in too frequent rejection or acceptance of the null hypothesis. Also, regression parameter estimates, the intercepts and the regression coefficients are inaccurate. When errors are uncorrelated, a Durbin-Watson test is

utilized. The Durbin-Watson test null hypothesis is that autocorrelation=0. The autocorrelation statistic, r , ranges in value from -1 to +1 with +1 being perfect correlation.

After these hypotheses were accepted or rejected, correlations between age and potential predictors by bone were calculated to indicate which measurement(s) might provide significant regression equations for the prediction of age. The significance level for entry into the stepwise model is $\alpha=0.15$. After selection, a variable will stay in the model only if the new model produces an F-value significant at $\alpha=0.10$. After selecting predictors with the stepwise model, the regression model was rerun to include all selected predictors. Any predictors in this model with regression coefficients that were not significant at an α of .05 were then dropped and the model rerun once more to create an equation to help estimate age-at-death as well as the coefficient of determination (r^2). The r^2 represents the amount of variation that can be accounted for by the proposed statistical model while the r^2 adjusted considers the number of independent variables in the model (Madrigal 1998). All models were formulated by bone for both males and females ($n=39$) and by bone separately for males ($n=22$) and females ($n=17$).

In addition, a discriminant function analysis was utilized to determine if sex could be assessed based on the collected histomorphometrics. A discriminant function analysis is a method to best distinguish between different subgroups based on a single combination of variables (Kirkwood 1988). For the purposes of this research, sex is used as the categorical variable and the histomorphometrics as the predicting sub-variables. This analysis was run for all vault bones jointly and then separately for the frontal, parietal and temporal.

Because the group means of the individuals in the discriminant function are based on the observations from the groups themselves, there is a bias towards a higher (and often inaccurate)

rate of classification. To account for this, a jack-knife classification of observations is performed. This essentially involves the process of assigning an individual to each group, in this case either male or female, without using that individual in determining the group means (Manly 2005).

A stepwise regression model was created to summarize which sub-variables from the three bones provide the most accurate discriminate function.

Intra-observer error

Fifteen individuals were selected using a table of random numbers and all variables (for each bone) were re-measured in order to evaluate and account for observer error. These two sets of observations were compared via the student's T-test for paired samples to ensure the absence of error in data collection and measurement. This was done to ensure repeatability of the histomorphometric observations collected by the author on two separate dates (six months apart).

Chapter 4: Results

Internal versus external tables

H₀₁ states that there are no significant differences in the mean values for each sub-variable in the internal and external tables. Based on the resulting p-values (Table 4.1), H₀₁ cannot be rejected at the Bonferroni adjusted alpha or at the original criterion. There are no significant differences between the internal and external means any of the sub-variables.

Males versus Females

H₀₂ states that there are no significant differences in the mean values for each sub-variable between males and females. Results demonstrate significance differences for certain sub-variables by sex for each bone (Table 4.2); therefore H₀₂ is rejected. Because of these findings, males and females were analyzed both together and separately in subsequent statistical tests.

Table 4.1 Repeated measures ANOVA to compare the mean values for sub-variable by table (internal and external)

Variable	Minimum	Maximum	Mean	P-value
OA (nm ²)	43720.85	107732.29	63672.01	0.916
OP (nm)	797.77	1279.12	999.37	0.689
HCA (nm ²)	170.46	292.93	216.96	0.465
HCP (nm)	1.44	2.11	1.78	0.589

Table 4.2 Significant (p-value) differences in sub-variable means by sex by bone

	ExtThick	OA	OP	ODMAX	ODMIN	HCA	HCP	HCMAX	HCMIN
Frontal	-	-	-	-	-	-	-	-	0.0086
Parietal	0.0003	-	-	-	-	0.0001	0.044	0.0024	-
Temporal	NA	-	-	0.0027	-	-	-	-	-

Differences between bones

A Tukey-Kramer Honestly Significantly Different (HSD) post hoc test looked for differences in the means for each male and female sub-variable between the frontal, parietal, and temporal bones (Table 4.3). H_{03} states that there are no significant differences between the means of each sub-variable by bone. H_{03} is rejected because there are numerous significant differences between the means of each sub-variable by bone.

Females: The means for each sub-variable were not significantly different between the frontal and the parietal bones. However, there were significant differences between two specific sub-variables in the frontal and temporal as well as the five sub-variables in the parietal and temporal.

Males: The means for each sub-variable were not significantly different between the parietal and temporal bones. There were five significance differences in means of the sub-variables of the frontal and temporal and six significance differences in means of the sub-variables of the frontal and parietal.

Independence of Observations

One of the primary assumptions of regression analyses is the independence of observations. Multiple observations taken from one single sample are likely correlated. The test for auto-correlation in observations was significant for all three bones (Table 4.4). Therefore, the appropriate approach to the regression analysis is to use aggregated data or data averaged by sample by bone.

Correlations of Age with Variables by Bone and by Sex

Measurement data for males and females was aggregated per bone. Multivariate statistical analyses determined correlations between each potential predictor and age. The Pearson Correlation Coefficient and p-value were calculated for every sub-variable by sex and by bone (Table 4.5).

Table 4.3 Significant (p-value) differences in sub-variable means by bone

P-values	ExtThick	OA	OP	ODMAX	ODMIN	HCA	HCP	HCMAX	HCMIN
Female F:T	NA	-	0.0082	0.0026	-	-	-	-	-
Male F:T	NA	-	0.0446	-	-	0.0013	0.0304	0.0014	0.0197
Female F:P	-	-	-	-	-	-	-	-	-
Male F:P	-	0.0145	0.0138	0.0099	-	0.0006	0.0070	0.0057	-
Female P:T	NA	-	0.0058	0.0108	-	0.0007	0.0067	0.0045	-
Male P:T	NA	-	-	-	-	-	-	-	-

F-Frontal; P-Parietal; T-Temporal

Table 4.4 Test for independence of observations

	Durbin-Watson	r	p-value
Female F	0.246	0.87	<0.0001
Male F	0.433	0.78	<0.0001
Female P	0.277	0.86	<0.0001
Male P	0.381	0.81	<0.0001
Female T	0.259	0.87	<0.0001
Male T	0.393	0.80	<0.0001

F-Frontal; P-Parietal; T-Temporal

Table 4.5 Correlation (p-value) of age with measurement by bone and by sex. *Significant values at alpha=0.05.

	Female F	Male F	Female P	Male P	Female T	Male T
ExtThick	0.2642 (0.3227)	-0.2975 (0.2027)	0.356 (0.1764)	-.0443 (0.8570)	NA	NA
OA	-0.273 (0.2725)	-0.153 (0.5071)	0.123 (0.6269)	-0.270 (0.2491)	-0.007 (0.9791)	-0.469 (*0.0320)
OP	-0.218 (0.3850)	-0.183 (0.4271)	0.055 (0.8281)	-0.258 (0.2723)	-0.022 (0.9300)	-0.410 (0.0646)
ODMAX	-0.234 (0.3502)	-0.193 (0.4030)	0.115 (0.6498)	-0.162 (0.4961)	-0.073 (0.7745)	-0.373 (0.0956)
ODMIN	-0.259 (0.2999)	-0.073 (0.7532)	-0.0214 (0.9330)	-0.354 (0.1259)	0.025 (0.9210)	-0.457 (*0.0371)
ODRATIO	0.040 (0.8763)	0.373 (0.096)	-0.199 (0.4277)	-0.084 (0.7256)	0.146 (0.5632)	-0.063 (0.7868)
HCA	-0.225 (0.3693)	-0.327 (0.1477)	-0.080 (0.7919)	-0.152 (0.5231)	-0.083 (0.7442)	-0.345 (0.1216)
HCP	-0.215 (0.3927)	-0.324 (0.1503)	-0.094 (0.7475)	-0.177 (0.4550)	-0.112 (0.6590)	-0.333 (0.1403)
HCMAX	-0.172 (0.4951)	-0.323 (0.1530)	-0.074 (0.5518)	-0.265 (0.2588)	-0.130 (0.6078)	-0.354 (0.1152)
HCMIN	-0.338 (0.1706)	-0.3697 (0.0990)	-0.077 (0.9299)	-0.213 (0.3674)	-0.104 (0.6816)	-0.194 (0.4002)
HCRATIO	-0.230 (0.3595)	0.101 (0.6641)	0.043 (0.3435)	0.202 (0.3921)	-0.004 (0.9887)	0.480 (*0.0278)

External Table Thickness: For males, external table thickness of the frontal bone was negatively correlated with age though for females there was a positive correlation. For males, the external table thickness of the parietal bone was also negatively correlated with age. However, for females, there was a positive correlation with age. None of these correlations were significant. External table thicknesses could not be measured in the temporal bone. There was no clear and consistent delineation between diploe and the internal and external tables.

Secondary Osteon Area and Perimeter: Osteon area and osteon perimeter are both negatively correlated with age for both males and females and for all bones except for the female parietal. None of these correlations were significant.

Secondary Osteon Maximum and Minimum Diameters: Maximum and minimum diameters of secondary osteons are negatively correlated with age except for in the female parietal (maximum diameter) and the female temporal (minimum diameter). There is a significant correlation between age and minimum secondary osteon diameter in the male parietal and in the male temporal.

Secondary osteon diameter ratio is negatively correlated with age for the female parietal bone. Secondary osteon diameter ratio (minimum/maximum) is positively correlated with age for the male frontal, parietal and temporal bones as well as the female frontal and temporal bones. It is significantly correlated with age in the male frontal bone.

Haversian Canal Area and Perimeter: Secondary osteon Haversian canal areas and diameters are negatively correlated with age for male and female neurocranial elements. However, there are no significant correlations with age.

Haversian Canal Maximum and Minimum Diameters: Haversian canal maximum and minimum diameters are negatively correlated with age for all bones in both males and females. None of these correlations are significant.

Haversian canal diameter ratio is positively correlated in all instances except for the female frontal and temporal. It is significantly correlated with age only in the male temporal.

Stepwise Regression

All measurements were used in linear regression via a stepwise selection procedure to determine which measurements or predictors to retain; parameter estimate tables test the significance of individual predictors and defines the regression equation to help estimate age.

Sex-Pooled equations:

Frontal Bone: One sub-variable met the significance level of $\alpha=0.15$ – Haversian canal minimum diameter (Figure 4.1). The r^2 is 0.13 and the standard error is 9.44. The prediction expression created is as follows:

$$\text{Age} = 56.32 - 4.69(\text{hcmin})$$

Parietal Bone: No sub-variables met the significance level of $\alpha=0.15$ for entry into the model.

Temporal Bone: One sub-variable met the significance level of $\alpha=0.15$ – osteon area (Figure 4.2). The r^2 is 0.072 and the standard error is 9.646. The prediction expression created is as follows:

$$\text{Age} = 47.32 - 0.000223(\text{OA})$$

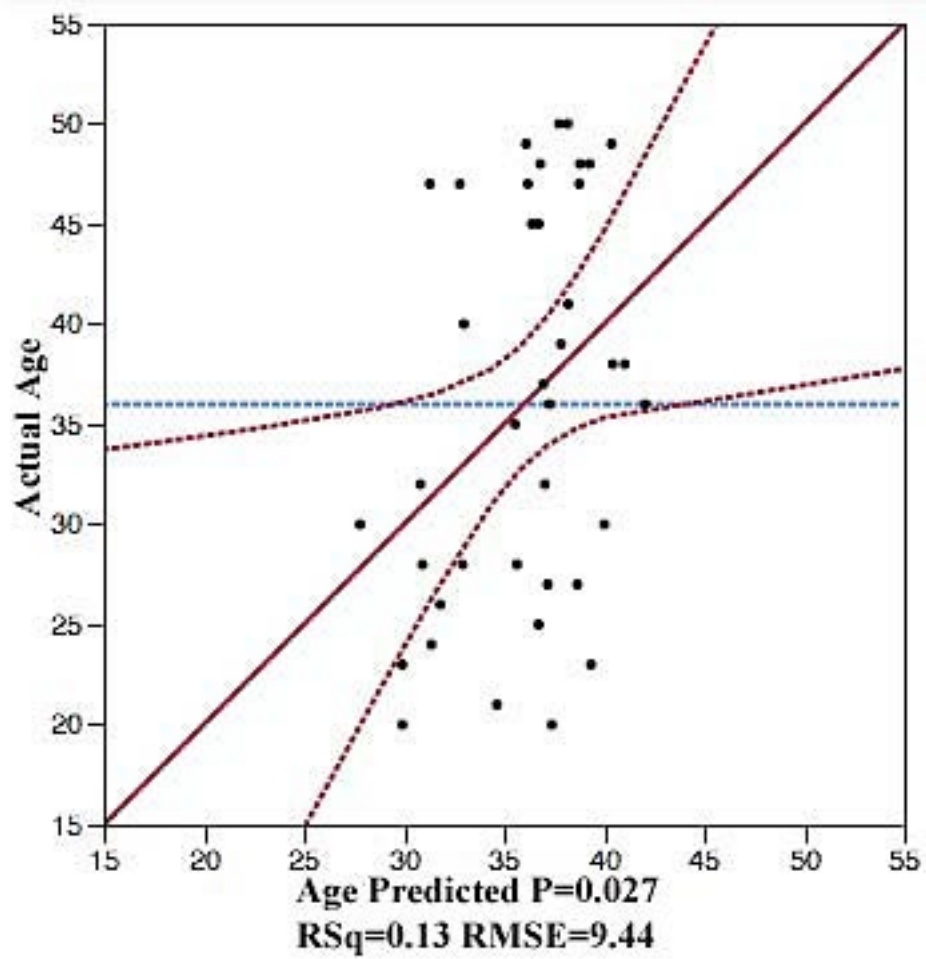


Figure 4.1: Sex-Pooled Actual by Predicted Age (in years) Plot Frontal Bone. RSq refers to the coefficient of determination and RMSE is the standard error.

(solid red: line of fit, dotted red: 0.5 significance curve, dotted blue: line of mean)

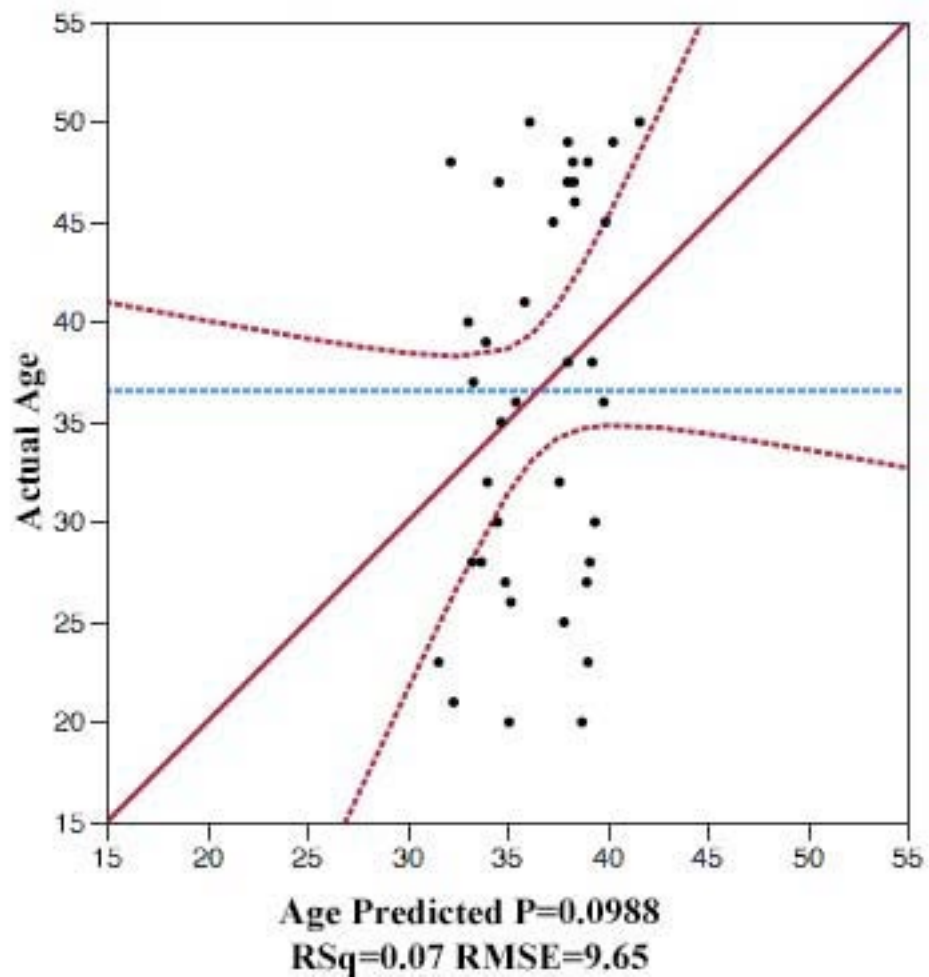


Figure 4.2: Sex-Pooled Actual by Predicted Age (in years) Plot of Temporal Bone. RSq refers to the coefficient of determination and $RMSE$ is the standard error.

(solid red: line of fit, dotted red: 0.5 significance curve, dotted blue: line of mean)

Sex Specific equations:

Female Frontal Bone: No sub-variables met the significance level of $\alpha=0.15$ for entry into the model.

Male Frontal Bone: One sub-variable met the significance level of $\alpha=0.15$ – osteon diameter ratio (Figure 4.3). The r^2 is 0.139 and the standard error 9.79. The prediction expression created is as follows:

$$\text{Age} = -2.041 + 62.34(\text{odratio})$$

Female Parietal Bone: No sub-variables met the significance level of $\alpha=0.15$ for entry into the model.

Male Parietal Bone: One sub-variable met the significance level of $\alpha=0.15$ for entry into the model – minimum osteon diameter (Figure 4.4). The r^2 is 0.125 and the standard error is 9.744.

$$\text{Age} = 70.197 - 0.1799(\text{odmin})$$

Female Temporal Bone: No sub-variables met the significance level of $\alpha=0.15$ for entry into the model.

Male Temporal Bone: One sub-variable met the significance level of $\alpha=0.15$ – Haversian canal diameter ratio (Figure 4.5). The r^2 is 0.23 and the standard error is 9.15 The prediction expression created is as follows:

$$\text{Age} = -14.052 + 68.074(\text{hcratio})$$

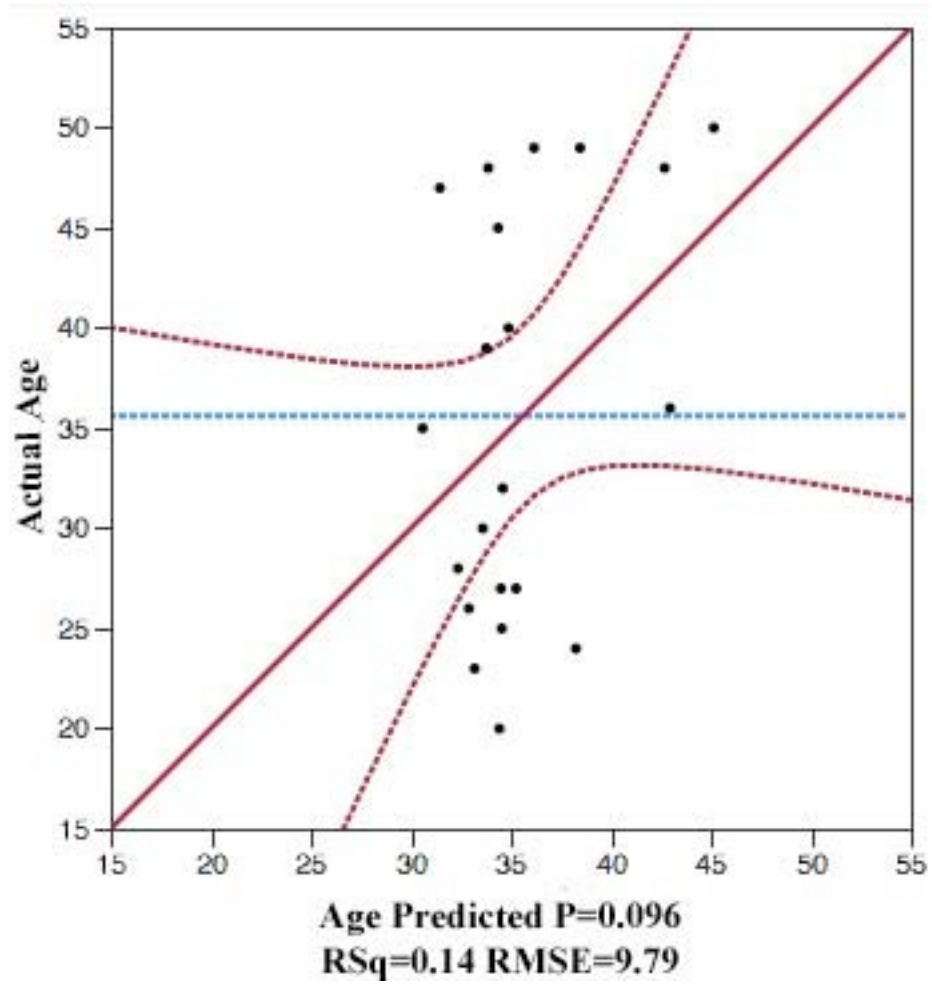


Figure 4.3: Actual by Predicted Age (in years) Plot of Male Frontal Bone. RSq refers to the coefficient of determination and RMSE is the standard error.

(solid red: line of fit, dotted red: 0.5 significance curve, dotted blue: line of mean)

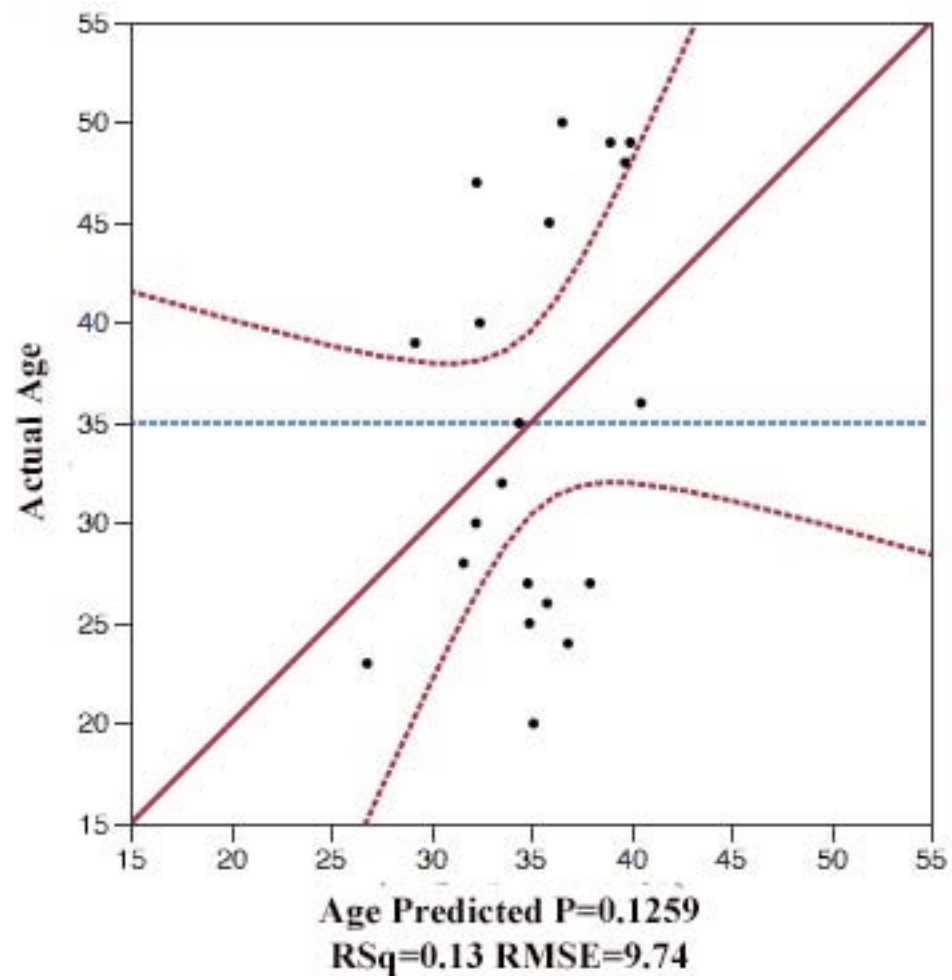


Figure 4.4: Actual by Predicted Age (in years) Plot of Male Parietal Bone. RSq refers to the coefficient of determination and RMSE is the standard error.

(solid red: line of fit, dotted red: 0.5 significance curve, dotted blue: line of mean)

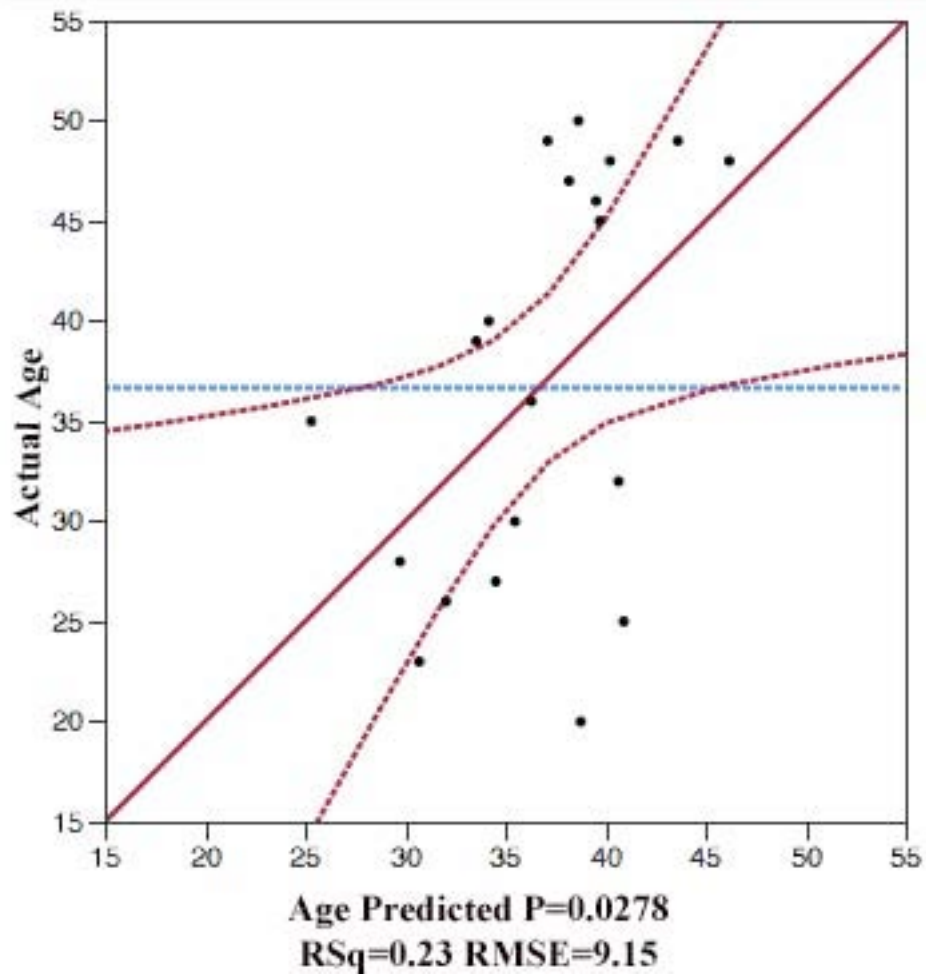


Figure 4.5: Actual by Predicted Age (in years) Plot of Male Temporal Bone. RSq refers to the coefficient of determination and RMSE is the standard error.

(solid red: line of fit, dotted red: 0.5 significance curve, dotted blue: line of mean)

Discriminant Function Analysis

A jack-knifed discriminant function analysis was run separately for the frontal, parietal and temporal bones and then again to include all three bones to determine how accurate the sub-variables are at “discriminating” between subgroups and predicting which subgroup (male or female) each individual belongs to.

Frontal: The jack-knifed discriminant function analysis for sex for the frontal bone produced the following actual rows by predicted columns matrix:

	F	M	% Correct
F	8	8	50
M	4	16	80
Total	12	24	67

Out of the sixteen females in the tested sample, eight were misclassified as males. Out of the twenty males in the tested sample, four were misclassified as females.

Parietal: The jack-knifed discriminant function analysis for sex for the parietal bone produced the following actual rows by predicted columns matrix:

	F	M	% Correct
F	6	10	38
M	11	9	45
Total	17	19	42

In this example, ten of the sixteen females were misclassified as males while eleven of the twenty males were misclassified as females.

Temporal: The jack-knifed discriminant function analysis for sex for the temporal bone produced the following actual rows by predicted columns matrix:

	F	M	% Correct
F	10	8	56
M	6	15	71
Total	16	23	64

When using histomorphometrics from the temporal bone to predict sex, eight of the eighteen females were misclassified as males while six of the twenty-one males were misclassified as females.

All bones: The jack-knifed discriminant function analysis for sex for all three neurocranial elements combined produced the following actual rows by predicted columns matrix:

	F	M	% Correct
F	12	3	80
M	2	18	90
Total	14	21	86

When the frontal, parietal and temporal are considered together, only three of the fifteen females were misclassified as males. Only two of the twenty males were misclassified as females. This discriminant function utilized a combination of sub-variables (Table 4.6) from all three bones to create the classification matrix.

Intra-Observer Error

The resulting p-values (Table 4.7) from the student t-test demonstrate that this method of data collection and results are replicable.

Table 4.6 Sub-variable selection and related p-values for neurocranial sexing discriminant function

Variable	Bone	F-ratio	p-value
hcratio	frontal	3.074	0.024
OA	frontal	3.449	0.013
hcmx	parietal	3.803	0.007
hcratio	parietal	4.279	0.003
HCA	parietal	4.546	0.002
odmin	parietal	4.826	0.001
odratio	parietal	5.201	0.001
hcmx	temporal	5.988	0.000
hcmin	temporal	6.390	0.000
OA	temporal	6.731	0.000
HCP	parietal	7.590	0.000
hcmin	parietal	7.521	0.000

Table 4.7 Test and resulting p-values for intra-observer error

P-values	Frontal	Parietal	Temporal
OA	0.9770	0.9047	0.9443
OP	0.9241	0.8250	0.9873
odmax	0.7964	0.9144	0.5726
odmin	0.6536	0.7919	0.4390
HCA	0.4997	0.6902	0.6470
HCP	0.5620	0.5480	0.4852
hcmx	0.8812	0.3427	0.8962
hcmin	0.8581	0.4648	0.5458

Chapter 5: Discussion

Two of the essential facets of the responsibilities of a forensic anthropologist are to estimate age-at-death and sex. When traditional macro-bone or dental aging methodologies are not possible, bone and dental histology have become promising alternatives. To utilize the full potential of bone histomorphometrics, it is necessary to study the microscopic features of the entire skeleton. The majority of past research (Ahlqvist and Damsten 1969; Ericksen 1991; Kerley 1965; Kerley and Ubelaker 1978; Thompson 1979) focuses on the long bones while this project considers three separate vault bones.

The histomorphometric assessment of neurocranial elements as they relate to age-at-death and sex estimation revealed several unique findings including the need for sex and bone-specific analyses. An examination of each sub-variable as it relates to age as well as the resulting regression equations for this research and comparatively to previous studies offers an impetus for future research.

With respect to the goals of this research, the following questions discuss the relationship of age and sex estimation to neurocranial histomorphometrics:

- 1. Are there significant differences in the mean values for each sub-variable in the internal and external tables?**

There are no significant differences in the histomorphometrics of the internal and external tables of the neurocranial elements used in this study. Bone remodeling occurs on both the periosteal and endosteal layers of bone. These layers line both cortices of the neurocranial elements. Therefore, it is not unexpected that the mean values of the sub-variables are similar between the inner and outer bone tables.

2. Are there significant differences in the mean values for each sub-variable between males and females?

There are significant differences between male and female histomorphometrics. These include the frontal sub-variable of Haversian canal minimum diameter, the parietal sub-variables of external table thickness, Haversian canal area, Haversian canal perimeter, and Haversian canal maximum diameter, and the temporal sub-variable of secondary osteon maximum diameter. Though these values are not consistent per bone, the results suggest sex specific rates of bone modeling and remodeling.

One interesting finding is that there were no significant differences in external table thickness between sexes in the frontal and temporal bone but there were significant differences in the parietal bone. These results disagree with Clark (1987) who did not observe significant sex differences in the parietal. They also do not agree with Curtis' (2003) findings that sex does influence the amount of variation in external table thickness of the frontal.

As stated in Curtis' research, this may be due to differences in measurement methodology as well some indistinct boundaries between the external table and the diploe. Adipocyte-filled lumen extended from the diploe into the external table in some thin-sections, though they were not present in all instances and never uniform in appearance and distribution. The presence of these lumen may have affected the accuracy of external table thickness measurements.

This important finding demonstrates the necessity for sex-specific analyses and equations when considering bone histology as it relates to age at death estimations and related research. It also establishes a need for additional investigation into the differences between male and female bony microstructure and how and why such patterns of modeling and remodeling as well as

aging and mechanical factors affect said microstructure. Of interest is which decade(s) of life significant sex differences in sub-variables become more prominent.

Larger sample sizes for both younger and older individuals can work to isolate when this variation appears and when it becomes most prevalent. This may shed light on the cause behind such significant variation whether it is hormonal, biomechanical or related to the size and function of the neurocranial element itself. It may also aid in producing more accurate sex-specific and age-at-death regression equations based on the sub-variables.

3. Are there significant differences in the mean values for each sub-variable between bone by sex?

Mean sub-variable measurements are significantly different between bones when controlling for sex. The frontal, parietal and temporal all form via intramembranous ossification but each element is structurally and morphologically different and because of this, are uniquely modeled and remodeled. Research has demonstrated the need to separately consider long bone elements when examining microstructure (sources). This proves to be true for the flat bones of the cranium as well and important for future research designs.

A precursory microscopic observation of the traits for each thin-section demonstrated a large amount of variability in features and osseous organization (Figures 5.1 and 5.2). For males, the parietal and temporal bones are more similar while for females the frontal and parietal bones are more alike. This finding is difficult to explain but may possibly be related to the differences in male and female bone integrity and muscle attachment sites and strain near the sampling areas. Additional inquiries are necessary to understand these results.

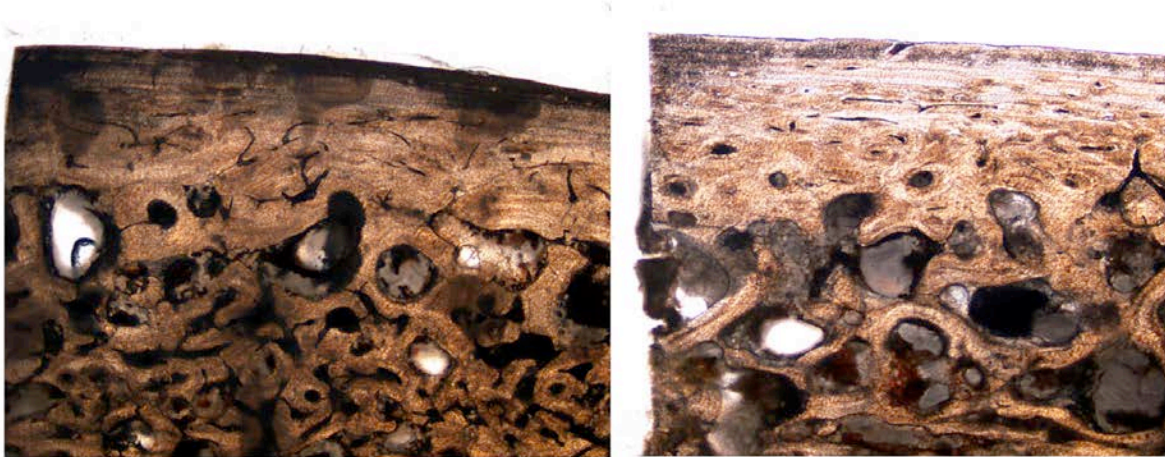


Figure 5.1: External table views of female (28 years) frontal (left) and male (32 years) frontal (right). Note the visible size difference in the thicknesses of the external tables. 1.6x/0.05 magnification.

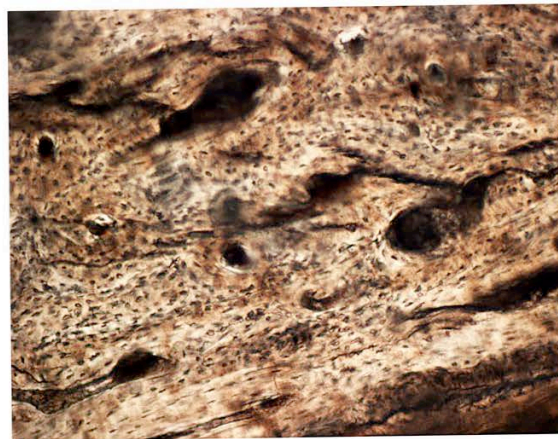


Figure 5.2: Variation in osteonal appearance in male (60 years) frontal (top), parietal (center) and temporal (bottom). Note that the size and shape of the secondary osteons and Haverisan canals as well as the organization of lamellar bone varies from one both to another. 1.6x/0.05 magnification.

4. Are there significant trends in the correlations between sub-variables and age?

Of the eleven sub-variables measured for the frontal, parietal and temporal bones, there are few significant associations with age. However, definite trends are evident in the correlations of sub-variable with age. These trends are comparable to past research results involving the neurocranial histomorphometrics (Clarke 1987; Cool et al. 1995; Curtis 2003). The majority of the sub-variables for this research were chosen based on Curtis' findings to see if those results for the frontal bone were reproducible in this study and if those results are similarly echoed when examining the parietal and temporal bones.

External Table Thickness: External table thicknesses in the male frontal and parietal bones are negatively correlated with age. However, the differences are not significant and therefore not reliable for estimating age.

These findings agree with those of Cool et al. (1995) who discerned a significant decrease in the outer table thickness with increased age in the occipital. They also support the observations of Clarke (1987) with the parietal bone as well as those of Curtis (2003) involving the frontal bone. However, the correlation between external thickness and age is positive for females in both the frontal and parietal. This could be due in part to the lack of older-aged individuals in the sample. Previous research indicates that females begin to increasingly lose cortical bone thickness in later decades (Thompson 1980). Thompson (1980) found that between the fifth and eighth decades of life, females lose up to 30% cortical thickness.

The changes in external table thicknesses (for the present age groups) are similar to that of previous neurocranial studies as well as long bone studies (Thompson 1979, 1980), suggesting that cortical remodeling in the neurocranium is comparable to that in postcranial elements.

Secondary Osteons: Secondary osteon area, secondary osteon perimeter and secondary osteon maximum diameter are all negatively correlated with age independent of sex or bone except for the female parietal. As age increases, the mean values of osteon area, perimeter and maximum diameter decrease. These results support Curtis (2003) who also found negative correlations between these sub-variables and age. The relationships discovered, though, are not as significant as those in Curtis'. Curtis' (2003) sample was predominately comprised of individuals over sixty years of age. Though the current sample has a wider age distribution, such a composition with only $n=39$, may have introduced that much more variability and subsequently weakened potential significant relationships.

In the male temporal bone, age is significantly correlated to secondary osteon area and minimum osteon diameter. Though general observations demonstrated a great deal of intra- and inter-bone variation for the temporal, this bone has never before been utilized in research and the results of this study merit it additional attention.

Haversian Canals: Secondary Haversian canal area, perimeter and maximum and minimum diameters are also negatively correlated with age regardless of sex or bone. These correlations are not significant but are similar to Curtis' (2003) findings. These sub-variables may not provide the most accurate and replicable measurements due to small size and the overall amount of variation and non-uniformity.

5. Are there significant relationships (p-values) between sub-variables and age? And can regression equations be created to estimate age at death?

Regression equations utilizing the sub-variables to estimate age-at-death were created for the male frontal, parietal and temporal as well as for the sex-pooled frontal and temporal bones. There were no significant correlations between sub-variables and age for the female frontal, parietal or temporal bones or for the sex-pooled parietal bone. The resulting r^2 values as well as the standard errors for the regression equations are displayed in Table 5.1. These values are relatively weak indicating that these histological sub-variables are not good predictors of age.

Confounding the potential unreliability of the equations is that most of the variables that were significant enough to remain in the regression model have lower p-values in the test for intra-observer error. These lower values indicate the poorer replicability of these measurements that is likely related to their small size. This fact can affect the reliability and accuracy of the prediction equations. Future research will focus on whether a successful reproduction of similar regression equations utilizing the same variables can be achieved.

Table 5.1 R-values and standard errors for regression equations

Sex	Bone	r-squared	standard error
Pooled	Frontal	0.130	9.44 years
Pooled	Temporal	0.072	9.65 years
Male	Frontal	0.139	9.79 years
Male	Temporal	0.230	9.15 years
Male	Parietal	0.125	9.74 years

In comparison to previous age-at-death estimation regression equations, the male frontal bone model is slightly stronger than that given by Curtis (2003). For Curtis' male frontal bone regression equation, the r^2 value is 0.118, the adjusted r^2 value is 0.097, with standard error of 13.755 years. This is similar to the findings of this research for the male frontal bone where r^2 is 0.139 and the standard error is 9.79 years. Curtis' (2003) results for the frontal bone are similar to those found in this research for the parietal ($r^2=0.125$; standard error=9.74 years) and temporal ($r^2=0.23$; standard error 9.15). However, the sub-variables used as predictors are different between these two equations. Curtis' use of external table thickness and osteon population density may actually create a more reproducible model even if the initial statistical results are weaker.

In this study, a sex-pooled regression equation was created for the frontal bone to estimate age. The resulting statistics are weak in comparison to those of Curtis (2003). Curtis' sex-pooled regression equation produced as r^2 value of 0.260 and a standard error of 13.132 years. In this study, the sex-pooled regression equation produced an r^2 value of 0.13 and a standard error of 9.44 years. The age distributions for both this study and Curtis' (2003) are slightly skewed towards younger and older individuals, respectively. This bias is very likely affecting the reliability and strength of the regression equations.

Additional research is required to re-examine the findings in this study and to expand upon the research design. Since osteon population density has been found to have a strong association with age (Clarke 1987; Cool 1995; Curtis 2003), this sub-variable should be included in the measurements. It was not incorporated into the current research because of the varying size and shape of the procured bone samples. Due to the disorganization of the histological structures

themselves, using a circular field similar to Kerley's (1965) method would not capture enough of that structure to measure and be representative of the entire thin-section. The use of bone cores would ensure a more standard area of bone necessary for histomorphometric analysis.

As mentioned, more focus will be given to the potential utility of the temporal bone histology. Prior to this study, it was never examined to estimate age-at-death. Females and males should continue to be analyzed separately and larger sample sizes with an even age distribution are necessary. This research may have suffered from being a smaller sample (n=39) but did address a younger demographic that has previously been absent from similar studies. Past research (Clarke 1987; Cool et al. 1995; Curtis 2003) relied heavily on a sample of older individuals. By addressing these issues, the results may provide more support for utilizing neurocranial histomorphometrics in estimating age-at-death.

6. Can histomorphometrics help to estimate sex?

A discriminant function analysis to estimate sex was created for all three neurocranial bones in both joint and separate models. When considering histomorphometrics of the frontal, parietal and temporal individually, the analysis does not demonstrate a reliable method to discriminate between sex sub-groups. The rates of correct classification vary not only by bone but also by sex (Table 5.2).

These findings are likely due to the age demographics of the sample and what is known about age-related microscopic changes in males and females. As mentioned earlier, Thompson (1980) indicates that with increasing age, females experience a much more significant decline in

Table 5.2 Discriminant function classification results

Bone	# of Males	Males misclassified	# of Females	Females misclassified	% Correct Classification
Frontal	20	4	16	8	67%
Parietal	20	11	16	10	42%
Temporal	21	6	18	8	64%
All	21	2	15	3	86%

cortical thickness and bone mineral mass that do males. Females also experience more significant age-related changes in Haversian canal areas than males (Thompson 1980). Because the current sample did not include individuals over the age of fifty, these differential fluctuations between the sexes may not yet be as strongly evident.

However, when the frontal, parietal and temporal are considered in a single analysis, the discriminant function is very accurate at classifying males and females – 90% correct classification for males and 80% correct classification for females. This finding merits additional attention because of such potential in estimating sex. With the addition of more discriminating microscopic sub-variables and larger and broader age ranges, the reliability of this method may prove to be even stronger and thus particularly useful in helping identify unknown remains.

Chapter 6: Conclusions

This research tests new methodologies for estimating age and sex for the purposes of the biological profile. It provides an additional tool for anthropologists and bioarchaeologists tasked with identifying unknown human remains that lack the gross morphological features required for conventional metric and non-metric approaches. These remains may be compromised by peri- or postmortem circumstances and may be highly fragmented or commingled.

This study utilized three separate neurocranial elements procured at autopsy from thirty-eight individuals of known age, sex and ancestry and applied histomorphometrics to develop new techniques to estimate age and sex. The demographics focused on individuals under the age of fifty, a sample previously under-represented in the literature. The following significant findings were made:

- There are no differences in the histomorphometrics between the internal and external tables of the frontal, parietal and temporal bones.
- There are significant differences in the sub-variable mean values between males and females. Sex specific analyses are necessary.
- There are significant differences in the sub-variable mean values between bone when controlling for sex.
- The correlations of sub-variable mean values and age are similar to the trends found in previous neurocranial studies.
- These correlation trends are additionally comparable to long bone histomorphometric research indicating similar processes of cortical remodeling.

Both sex-specific and sex-pooled regression equations were generated for age estimation. Regression equations for the sex-pooled frontal and temporal bone as well as for the male frontal, parietal and temporal bone were produced based on significant correlations between microscopic sub-variables and age-at-death. The resulting r^2 values range from 0.072 – 0.230 with standard errors from 9.15 – 9.79 years. This demonstrates that neurocranial microstructure undergoes age-related changes and that these changes have utility in estimating age-at-death. Though not as effective as histomorphometric methods established for postcranial elements, these equations nevertheless provide a means of estimating an aspect of the biological profile when traditional gross morphological aging techniques cannot be used.

This research also created a discriminant function analysis based on the collected histomorphometrics to estimate sex. Though previous studies have noted the need for sex-specific equations to estimate age, they have not addressed the possibility of using the microstructure itself to classify individuals as males or females. The discriminant function analysis utilized a combination of sub-variables from the frontal, parietal and temporal to estimate sex and did so with 80% correct classification for females and 90% correct classification for males. These results lend credibility to the prospect of using bone microstructure to estimate sex.

Neurocranial histomorphometrics offer an additional resource for partial estimation of the biological profile. The results of this study indicate the need for future research designs to fine-tune the age-prediction regression equations and to further investigate the sex estimation utility.

References

Acsadi, G. and Nemeskeri, J. 1970. History of human life span and mortality. Akademiai Kiado (Budapest).

Ahlqvist, J. and Damsten, O. 1969. A modification of Kerley's method for the microscopic determination of age in bone. *Journal of Forensic Science* 14:205-212.

Aiello, L.C., Molleson, T. 1993. Are microscopic ageing techniques more accurate than macroscopic ageing techniques? *Journal of Archaeological Science* 20:689–704.

Amprino, R. and Marotti, G. 1964. A topographic quantitative study of bone formation and reconstruction. In (H.J.J. Blackwood, Ed) Bone and Tooth, Proc. 1st European Symposium, Pergamon Press, London.

Ardizzoni, A. 2001. Osteocyte lacunar size-lamellar thickness relationships in human secondary osteons. *Bone* 28:215-219.

Ascenzi, A. and Bonucci, E. 1967. The tensile properties of single osteons. *The Anatomical Record* 158:375–386.

Ascenzi, A. and Bonucci, E. 1968. The compressive properties of single osteons. *The Anatomical Record* 161: 377–391.

Atkinson, P.J. 1965. Changes in resorption spaces in femoral cortical bone with age. *Journal of Pathology and Bacteriology* 89:173-178.

Baron, R, Vignery, A., Horowitz, M. 1984. Lymphocytes, macrophages and the regulation of bone remodeling. In (W.A. Peck, Ed) Bone and Mineral Research Annual, Elsevier, Amsterdam.

- Bartsiokas, A. 2002. Hominid cranial bone structure: A histological study of Omo 1 Specimens from Ethiopia using different microscopic techniques. *The Anatomical Record* 267:52-59.
- Bass, W.M. 1995. Human osteology: a laboratory and field manual. Columbia, MO: Missouri Archaeological Society.
- Beaupre, G.S., Stevens, S.S., Carter, D.R. 2000. Mechanobiology in the development, maintenance, and degeneration of articular cartilage. *Journal of Rehabilitation Research and Development* 37:145-151.
- Bell, L.S. 1990. Palaeopathology and diagenesis: an SEM evaluation of structural changes using backscattered electron imaging. *Journal of Archaeological Science* 85-102.
- Berryman H.E. and Symes, S.A.1998. Recognizing gunshot and blunt cranial trauma through fracture interpretation. In (Reichs K.J. Ed) *Advances in the identification of human remains*. Springfield: Charles Thomas Publisher 333-352.
- Bouvier, M. and Hylander, W.L. 1981. Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). *Journal of Morphology* 167:1-12.
- Britz, H.M., Thomas, C.D.L., Clement, J.G., Cooper, D.M.L. 2009. The relation of femoral osteon geometry to age, sex, height, and weight. *Bone* 45:77-83.
- Brooks, S.T. 1955. Skeletal age at death, the reliability of cranial and pubic age indicators. *American Journal of Physical Anthropology* 13:567-97.

Buikstra, J.E. and Ubelaker, D.H. 1994. Standards for Data Collection from Human Skeletal Remains: Proceedings of a seminar at The Field Museum of Natural History. Arkansas Archaeological Survey Research Series No. 44.

Burr, D.B. 1985. Symposium on current theoretical and experimental perspectives on bone remodeling mechanisms: Introductory comments. *Yearbook of Physical Anthropology* 28:207-210.

Carter, D.R. and Beaupre, G.S. 2001. Skeletal Form and Function. Mechanobiology of Skeletal Development, Aging, and Regeneration. Cambridge University Press.

Charles, D.K., Condon, K., Cheverud, J.M. and Buikstra, J.E. 1989. Estimating age at death from growth layer groups in cementum. In (Iskan and Kennedy, Eds) Age Markers in the Human Skeleton, Springfield: Charles C. Thomas: 277-316.

Clarke, D.F. 1987. Histological and radiographic variation in the parietal bone in a cadaveric population. Masters Thesis: University of Queensland, Australia.

Cool, S.M., Hendrikz, J.K., Wood, W.B. 1995. Microscopic age changes in the human occipital bone. *Journal of Forensic Sciences* 40:789-796.

Crowder, C. M. 2009. Histological Age Estimation. In (S. Blau and D.H. Ubelaker Eds) Handbook of Forensic Anthropology and Archaeology. World Archaeological Congress Research Handbooks in Archaeology. Left Coast Press: Walnut Creek, California. Pgs 222-235.

Crowder, C.M. and Stout, S.D. 2012 Bone Histology, An Anthropological Perspective. CRC Press.

Currey, J. D. 1959. Differences in the tensile strength of bone of different histological types. *Journal of Anatomy (London)* 93:87–95.

Currey, J. D. 1964. Some effects of ageing in human haversian systems. *Journal of Anatomy*. 98:69-75.

Currey, J. D. 1970. The mechanical properties of bone. *Clinical Orthopedics*. 73:210-231.

Currey, J.D. 1982. ‘Osteons’ in biomechanical literature. *Journal of Biomechanics* 15:717.

Currey, J.D. 2002. Bones: structure and mechanics. Princeton University Press, New Jersey.

Curtis, J. M. 2003. Estimation of age at death from the microscopic appearance of the frontal bone, Master's Thesis: Graduate School of the University of Indianapolis.

Dempster, W.T. and Enlow, D.H. 1959. Patterns of vascular channels in the cortex of the human mandible. *The Anatomical Record* 135:189-205.

Drusini, A. and Businaro, F. 1990. Skeletal age determination by mandibular histomorphometry. *International Journal of Anthropology* 5:235–243.

Duterloo, H. S. and Enlow, D. H. 1970. A comparative study of cranial growth in *Homo* and *Macaca*. *American Journal of Anatomy* 127: 357–367.

Endo, B. 1966. Experimental studies on the mechanical significance of the form of the human facial skeleton. *Journal of the Faculty of Sciences University of Tokyo* III:1–101.

Endo, B. 1970. Analysis of stresses around the orbit due to masseter and temporalis muscles respectively. *Journal of the Anthropological Society of Nippon* 78:251–266.

Enlow, D.H., and Brown, S.O. 1956-58. A comparative histological study of fossil and recent bone tissues: Parts I, II and III. *Texas Journal of Science* 7: 405-443; 9:186-214; 10:187-230.

Enlow, D.H. 1962b. Functions of the Haversian system. *American Journal of Anatomy* 110: 269-305.

Enlow, D.H. 1986. Normal craniofacial growth. In (Cohen, M.M.J. Ed) *Craniosynostosis: diagnosis, evaluation, and management*. New York: Raven Press. Pgs. 131–156.

Enlow DH. 2000. Normal craniofacial growth. In (Cohen, M.M.J. and MacLean, R.E., Eds) *Craniosynostosis: diagnosis, evaluation, and management*, 2nd ed. New York: Oxford University Press. Pgs. 35–50.

Ericksen, M.F. 1991. Histological estimation of age at death using the anterior cortex of the femur. *American Journal of Physical Anthropology* 84:171-179.

Ericksen, M.F., Aselrod, D.W., Melsen, F. 1994. *Bone Histomorphometry*. New York: Raven Press.

Evans, F.G. 1957. *Stress and strain in bone*. Springfield: Thomas.

Evans, F.G. 1973. *Mechanical properties of bone*. Springfield, IL, Charles C. Thomas.

Evans, F.G. and Lebow, M. 1951. Regional differences in some of the physical properties of the human femur. *Journal of Applied Physiology* 3:563-572.

Frost, H.M. 1963a. *Bone Remodeling Dynamics*. Charles C. Thomas: Springfield, IL.

- Frost, H.M. 1964. *The Laws of Bone Structure*. Springfield, Thomas.
- Frost, H.M. 1987. Secondary osteon populations. An algorithm for determining mean bone tissue age. *Yearbook of Physical Anthropology* 30:221-238.
- Frost, H.M. 1997. Obesity, and bone strength and “mass”: A tutorial based on insights from a new paradigm. *Bone* 21:211-214.
- Galloway, A. 1999. Fracture patterns and skeletal morphology: introduction and the skull. In (Galloway, A., Ed) *Broken bones: anthropological analysis of blunt force trauma*. Springfield, IL: Charles C Thomas. Pgs 63–80.
- George, D.R. 2003. Thin-section analysis of faunal remains: An internet resource Retrieved from http://archnet.asu.edu/archnet/uconn_extras/faunal/section.html and http://archnet.asu.edu/archnet/uconn_extras/faunal/catalog.html
- Gilbert, B.M. and McKern, T.W. 1973. A method for aging the female os pubis. *American Journal of Physical Anthropology* 38:31–38.
- Gustafson G. 1950. Age determination on teeth. *Journal of the American Dental Association* 41:45-54.
- Hall, B. K. 2005. *Bones and cartilage: Developmental and evolutionary skeletal biology*. Elsevier Academic Press, London.
- Heersche, J.N.M. 1978. Mechanism of osteoclastic bone resorption: A new hypothesis. *Calcified Tissue Research* 26:81-84.
- Hillson, S. 1996. *Dental Anthropology*. Cambridge: Cambridge University Press.

Horni, H. 2002. The Forensic Application of Comparative Mammalian Histology. Masters Thesis: Texas Tech University, Lubbock, TX.

Hughes-Fulford, M. 2004. Signal transduction and mechanical stress. *Science STKE*, Retrieved from: www.stke.org/cgi/content/full/sigtrans;2004/249/re12.

Hylander, W.L. 1981. Patterns of stress and strain in the macaque mandible. In (D.S. Carlson Ed.) *Craniofacial Biology*. Center for Human Growth and Development, Ann Arbor.

Hylander, W.L. 1987. Loading patterns and jaw movements during mastication in *Macaca fascicularis*: a bone-strain, electromyo-graphic, and cineradiographic analysis. *American Journal of Physical Anthropology* 72: 287–314.

Iscan, M.Y., Loth, S.R., Wright, R.K. 1984. Age estimation from the rib by phase analysis: white males. *Journal of Forensic Sciences* 29:1094-1104.

Jaffe, H.L. 1929. The structure of bone with particular reference to its fibrillar nature and the relation of function to internal architecture. *Archives of Surgery* 19:24-52.

Jaworski, Z.F.G. 1984. Lamellar bone turnover system and its effector organ. *Calcified Tissue International* 36:46-S55.

JMP Software Version 9.0.2. JMP 7.0 (SAS Institute Inc., Cary, NC).

Johnson, L.C. Morphologic analysis in pathology: The kinetics of disease and general biology in bone. In (H.M. Frost Ed) *Bone Biodynamics*, Little and Brown, Boston.

Jowsey, J. 1966. Studies of haversian systems in man and some animals. *Journal of Anatomy* 100:857-864.

Jowsey, J., 1977. Metabolic diseases of bone W.B. Saunders Co, London.

Junqueira, L.C., Carneiro, J., Kelley, R.O. 1998. Bone. In Basic Histology, 9ed, Appleton and Lange, Stanford, Connecticut, Pgs. 134-151.

Junqueira, L.C. and Carneiro, J. 2003. Bone. In (Foltin, J., Lebowitz, H., Boyle, P.J. Eds). Basic Histology: Text and Atlas, 10ed. McGraw-Hill Companies, Inc, Pgs.141-159.

Kerley, E.R. 1965. The microscopic determination of age in human bone. *American Journal of Physical Anthropology* 23:149-164.

Kerley, E.R. and Ubelaker, D.H. 1978. Revisions in the microscopic method of estimating age at death in human cortical bone. *American Journal of Physical Anthropology* 49:545-546.

Keough, N. 2007. Estimation of age at death from the microscopic structure of the femur. University of Pretoria Dissertation. Pretoria, South Africa.

Kimura, K. 1992. Estimation of age at death from second metacarpals. *Zeitschrift für Morphologie und Anthropologie* 79:169-181.

Kirkwood, B.R. 1988. Essentials of Medical Statistics. Blackwell Science.

Krogman, W.M. 1962. The human skeleton in forensic medicine. Springfield, Illinois: Thomas.

Krogman, W.M. and Isçan, M.Y. 1986. The Human Skeleton in Forensic Medicine, 2ed, Springfield.

Lacroix, P. 1971. The internal remodeling of bones. In (Bourne, H.C., Ed.) The biochemistry and physiology of bone. New York: Academic Press. Pgs. 119-144.

Lanyon, L.E. 1981. Osteoporosis and mechanically related bone modeling. In (J.Menczel, G.C. Robin, M. Makin, and R. Steinberg Eds) Osteoporosis. Proceedings of the International Symposium, Jerusalem Osteoporosis Center, John Wiley and Sons, Chichester.

Lanyon, L.E. and Bourn, S. 1979. The influence of mechanical function on the development and remodeling of the tibia. *Journal of Bone and Joint Surgery* 61A:263-273.

Laval-Jeantet AM, Bergot C, Carroll R, Garcia-Schaefer F. 1983. Cortical bone senescence and mineral bone density of the humerus. *Calcified Tissue International* 35:268-272.

Leeson, T.S. and Leeson, R.C. 1981. Specialized connective tissue: cartilage and bone. In Histology, 4ed, W.B. Saunders Company, Pgs. 137-164.

Lovejoy, C.O., Meindl, R.S., Pryzbeck, T.R., Mensforth, R.P. 1985. Chronological metamorphosis of the auricular surface of the ilium: A new method for the determination of adult skeletal age at death. *American Journal of Physical Anthropology* 68:15-28.

Madrigal, L.1998. Statistics for Anthropology. Cambridge University Press.

Manley, B.F.J. 2005. Multivariate statistical methods: a primer. CRC Press.

Martin, R.B. and Burr, D.B. 1989. Structure, function, and adaptation of compact bone. New York, New York: Raven Press.

Martin, D.L. 1991. Bone histology and paleopathology: methodological considerations. In (Ortner, D.J. and Aufderheide A.C., Eds) Human paleopathology: Current syntheses and future options. Washington: Smithsonian Institution Press., Pgs. 55-59.

Martin, R.B., Burr, D.B., Sharkey, N.A. 1998. *Skeletal Tissue Mechanics*. Springer-Verlag New York, Inc.

Martinez-Maza, C., Rosas, A., Garcia-Vargas, S. 2006. Bone paleohistology and human evolution. *Journal of Anthropological Science* 84: 77–81.

McKern T.W. and Stewart T.D. 1957. Skeletal age changes in young American males. Natick: Quartermaster Research and Development Command Technical Report EP-45.

Meindl, R.S., Lovejoy, C.O., Mensforth, R.P., Walker, R.A. 1985. A revised method of age determination using the os pubis, with a review and tests of accuracy of other current methods of pubic symphyseal aging. *American Journal of Physical Anthropology* 68:29–45.

Meindl, R.S. and Lovejoy, C.O. 1985. Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *American Journal of Physical Anthropology* 68: 57-66.

Monro, A. 1776. *The anatomy of the human bones, nerves and lacteal sac and duct*. Unknown publisher, Dublin.

Moodie, R.L. 1923. *Paleopathology*. Urbana: University of Illinois Press.

Mulhern, D.M. and Ubelaker, D.H. 2001. Differences in osteon banding between human and nonhuman bone. *Journal of Forensic Sciences* 46:220-222.

Nordin, M. and Frankel, V.H. 2001. *Basic Biomechanics of the Musculoskeletal System*. Lippincott Williams & Wilkins.

Opperman, L.A. 2000. Cranial sutures as intramembranous bone growth sites. *Developmental Dynamics* 219:472–485.

Ortner D.J. 1975. Aging effects on osteon remodeling. *Calcified Tissue Research* 18: 27-36.

Osbourne, D.L., Simmons T.L., Nawrocki, S.P. 2004 Reconsidering the auricular surface as an indicator of age at death. *Journal of Forensic Sciences* 49:1-7.

Ott, S.M. 1996. Theoretical and methodological approach. In (J.P. Bilezikian, L.G. Raisz, G.A. Rodan Eds) *Principles of Bone Biology*. San Diego: Academic Press.

Ousley, S.D. and Jantz, R.L. 2005. FORDISC 3.0: Personal Computer Forensic Discriminant Functions. University of Tennessee.

Paine, R.R. and Brenton, B.P. 2006. Dietary health does affect histological age assessment: An evaluation of the Stout and Paine (1992) age estimation equation using secondary osteons from the rib. *Journal of Forensic Sciences* 51:489-492.

Pauwels, F. 1965. *Gesammelte Abhandlungen zur Funktionellen Anatomie des Bewegungsapparates*. Springer, Berlin.

Pearson, K. 1917-1919. A study of the long bones of the English skeleton I: University of London, University College, Department of Applied Statistics, Company Research, Memoirs, Biometric Series X, chapters 1-4.

Petrtyl, M., Hert, J., Fiala, P. 1996. Spatial organization of the haversian bone in man. *Biomechanics* 29:161-169.

Peterson, J., and Dechow, P.C. 2003. Material properties of the human cranial vault and zygoma. *The Anatomical Record Part A* 274A:785–797.

Pfeiffer, S. 2000. Palaeohistology: Health and disease. In (Katzenberg, M.A. and Saunders, S.R., Eds.) *Biological anthropology of the human skeleton*. New York: Wiley-Liss. Pgs. 281-296.

Pfeiffer, S., Crowder, C., Harrington, L., Brown, M. 2006. Secondary osteon and Haversian canal diameters as behavioral indicators. *American Journal of Physical Anthropology*, 131:460-468.

Phenice, T.W. 1969. A newly developed visual method of sexing the *Os pubis*. *American Journal of Physical Anthropology* 30:297-302.

Powers, R. 1962. The disparity between known age and age as estimated by cranial suture closure. *Man* 83,84: 52-55.

Pratte D.G., Pfeiffer S. 1999. Histological age estimation of a cadaveral sample of diverse origins. *Canadian Society of Forensic Science Journal* 32:155-167.

Recker, R.R. 1983. *Bone histomorphometry: Techniques and interpretation*. CRS Press: Boca Raton.

Ribot, C., Tremollieres, F., Pouilles, J.M., Bonneu, M., Germain, F., Louvet, J.P. 1987. Obesity and postmenopausal bone loss: the influence of obesity on vertebral density and bone turnover in postmenopausal women. *Bone* 8:327-331.

Roberts, C. and Wakely J. 1992. Microscopical findings associated with the diagnosis of osteoporosis in paleopathology. *Journal of Osteoarchaeology* 2:23-30.

Robling, A.G., and Stout, S.D. 1999. Morphology of the drifting osteon. *Cells Tissues Organs* 164:192-204.

Robling, A.G., and Stout, S.D. 2000. Histomorphometry of human cortical bone: applications to age estimation. In (Katzenberg, M.A. and Saunders, S.R., Eds.) *Biological Anthropology of the Human Skeleton*. New York: Wiley-Liss Pgs. 187-213.

Ross, M.H., Reith, E.J., Romwell, L.J. 1989. *Histology: A text and atlas*. Baltimore: Williams and Wilkins.

Rubin, C.T. and Lanyon, L.E. 1982. Limb mechanics as a function of speed and gait: A study of functional strains in the radius and tibia of horse and dog. *Journal of Experimental Biology* 101:187-211.

Samson, C. and Branigan, K. 1987. A new method of estimating age at death from fragmentary and weathered bone. In (Boedington, A., Garland, A.N. and Janaway, R.C. Eds.) *Death, decay and reconstruction: Approaches to archaeology and forensic science*. Manchester, Manchester University Press. Pgs. 109-126.

SAS software, Version 9.2 of the SAS System for Windows. Copyright © 2010. SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

Scheuer L., Black S. 2000. *Developmental juvenile osteology*. San Diego: Academic Press.

Schwartz, J.H. 1995. *Skeleton keys: An introduction to human skeletal morphology, development and analysis*. Oxford: Oxford University Press.

- Sharpe, W. D. 1979. Age Changes in Human Bone: An Overview. *Bulletin of the New York Academy of Medicine* 55:757-773.
- Singer, R. 1953. Estimation of age from cranial suture closure: a report on its unreliability. *Journal of Forensic Medicine* 1:52-59.
- Singh, I.J., Tonna, E.A, Gandel, C.P. 1974. A comparative histological study of mammal bones. *Journal of Morphology* 144:421-438.
- Smith, J.W. and Walmsley, R. 1959. Factors affecting the elasticity of bone. *Journal of Anatomy* 93:503-522.
- Stewart, T.D. 1979. *Essentials of forensic anthropology*. Springfield, Illinois: Thomas.
- Stout, S.D. 1989. Histomorphometric Analysis of human skeletal remains. In (Kennedy, K.K. and Iscan, M.Y., Eds) *Reconstruction of life from the skeleton*. New York: Alan R. Liss. Pgs. 41-52.
- Stout, S.D. 1992. Methods of determining age at death using bone microstructure. In (Saunders, S.R. and Katzenberg, M.A., Eds.) *Skeletal biology of past peoples: research methods*. New York: Wiley-Liss. Pgs. 21-25.
- Stout, S.D. and Paine, R.R. 1992. Brief communication: histological age estimation using rib and clavicle. *American Journal of Physical Anthropology* 87:111-5.

- Stout, S.D. and Stanley, S.C. 1991. Percent osteonal bone versus osteon counts: the variable of choice for estimating age at death. *American Journal of Physical Anthropology* 86:515- 519.
- Suchey, J.M. and Katz, D. 1998. Applications of pubic age determination in a forensic setting. In (Reichs, K.J., Ed.) *Advances in the identification of human remains*. Springfield: Charles Thomas Publisher Pgs. 204-236.
- Tersigni, M.A. 2005. Serial long bone histology: inter- and intra-bone age estimation. Doctoral Thesis Knoxville, TN: University of Tennessee.
- Thompson, D.D. 1979. The core technique in the determination of age at death in skeletons. *Journal of Forensic Sciences* 24:902-915.
- Thompson, D.D. 1980. Age changes in bone mineralisation, cortical thickness, and Haversian canal area. *Calcified Tissue International* 31:902-915.
- Thompson, D.D. and Gunness-Hey, M. 1981. Bone mineral osteon analysis of Yupikinupiaq skeletons. *American Journal of Physical Anthropology* 55:1-7.
- Thompson, D.W. 1917. *On Growth and Form*. Cambridge University Press, London.
- Todd, T.W. 1920. Age changes in the pubic bone. The white male pubis. *American Journal of Physical Anthropology* 13:285–339.
- Todd, T.W. and Lyon, D.W. 1924. Endocranial suture closure its progress and age relationship. Part 1 -adult males of white stock. *American Journal of Physical Anthropology* 7:325-384.
- Todd, T.W. and Lyon, D.W. 1925. Cranial suture closure its progress and age relationship. Part 2-ectocranial closure in adult males of white stock. *American Journal of Physical Anthropology* 8:23-45.

Todd, T.W. and Lyon, D.W. 1925. Cranial suture closure its progress and age relationship. Part 3-endocranial closure in adult males of negro stock. *American Journal of Physical Anthropology* 8:47-71.

Tome, J. and De Morgan, C. 1853. Observations on the structure and development of bone. *Philosophical transactions of the royal society of London* 143:109-139.

Trammell, L.H. 2006. A Histological Examination of *Odocoileus virginianus* for Forensic Application. Masters Thesis, University of Tennessee.

Trammell, L.H., Marks, M.K., Klippel, W.E., Mileusnic-Polchan, D. 2008. Cranial Histomorphology: Species Identification and Age Estimation. *Proceedings of the American Academy of Forensic Sciences 60th Annual Scientific Meeting*.

Trehanne, R.W. Review of Wolff's Law and its proposed means of operation. *Orthopaedic Reviews* 10:35-47.

Ubelaker, D.H. 1989. Human skeletal remains: excavation, analysis, and interpretation. Washington DC: Taraxacum.

Washburn, S.L. 1948. Sex differences in the pubic bone. *American Journal of Physical Anthropology* 6:199-208.

Watanabe Y, Konishi M, Shimada M, Ohara H, Iwamoto S. 1998. Estimation of age from the femur of Japanese cadavers. *Forensic Science International* 98:55-65.

White, A.A., Panjabi, M.M., Southwick, W.O. 1977. The four biomechanical stages of healing. *Journal of Bone and Joint Surgery* 59A:188-192.

White, T.D. and Fokens, P.A. 2000. Human osteology. New York: Academic Press.

Wolff, J. 1892. Das Gesetz der Transformation der Knochen. A. Hirschwald, Berlin.

Woods and Ellis. 2000. *Bone*. Retrieved from <http://home.primus.com/au/rovellis/BONE.html>

Vita

Lindsay Hines Trammell was born November 21, 1981, in Greenville, South Carolina. She was raised in Simpsonville, South Carolina, and graduated Valedictorian from Hillcrest High School in 2000. From there, she attended the University of South Carolina Honors College in Columbia, South Carolina, and received her Bachelor of Arts degree in anthropology in 2004. She was accepted to the University of Tennessee-Knoxville anthropology program in August 2004 and earned her Masters of Arts in August 2006. She then entered the PhD program in physical anthropology at the University of Tennessee-Knoxville and advanced to candidacy in May of 2009. She received her Doctorate of Philosophy in May 2012.