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PERIOSTEAL VERSUS ENDOSTEAL SURFACE EQUATIONS

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Andrea L. Clowes

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Todd W Fenton
Major Professor's Signature

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A COMPARISON OF TWO METHODS OF AGE DETERMINATION USING HISTOMORPHOLOGY: PERIOSTEAL VERSUS ENDOSTEAL SURFACE EQUATIONS

By

Andrea L. Clowes

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ABSTRACT

COMPARISON OF TWO METHODS OF AGE DETERMINATION USING HISTOMORPHOLOGY:
PERIOSTEAL VERSUS ENDOSTEAL SURFACE EQUATIONS

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Establishing the age at death in a human remains case is a critical component in positive identification. Forensic techniques of bone histology, the microstructural analysis of tissue, are known to be accurate age estimators. This thesis is meant to explore the accuracy of the Hauser et al. method (1980) and compare it with the Kerley-Ubelaker method (1978). By directly comparing the two methods, the applicability of the equations of Hauser et al. are showcased next to those of Kerley-Ubelaker.

A sample of 30 femoral midshaft thin section slides (9 to 82 years of age at death) from the Dominican Republic was borrowed from Dr. Douglas Ubelaker at the National History Museum of Natural History. Age estimations were determined using the Kerley-Ubelaker method (KUA) (Kerley and Ubelaker, 1978) and the subperiosteal (HPA), endosteal (HEA), and average of endosteal/subperiosteal (HAA) equations of the Hauser method (1980). Paired t-tests were used to determine if there was a significant difference from the actual age at death for KUA, HPA, HEA, and HAA. Results revealed that the Hauser subperiosteal age estimate ($p=.05$) was the only accurate equation for the full sample. When analyzing the femoral thin sections of individuals older than 21 years at death, KUA, HPA, and HAA were all found to be accurate. Both sample sets indicated that Hauser endosteal age is an inaccurate age estimator with the Dominican Republic sample.

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Chapter 1: Introduction

Forensic anthropology is the application of the knowledge of skeletal biology to a medicolegal setting. In 1977, forensic anthropology achieved legal status with the establishment of the American Board of Forensic Anthropology sponsored by the Physical Anthropology Section of the American Academy of Forensic Sciences (AAFS) (Kerley, 1978). The goal of the forensic anthropologist is to examine human remains in order to extract as much information as possible leading to a positive identification or an explanation as to circumstances surrounding that individual's death. Beyond the identification of unknown human remains, research in forensic anthropology has been ongoing since the late 1800s. Research has focused on skeletal growth and development as it relates to age and is applicable to the reconstruction of the biological nature of prehistoric populations as well as the legal solution of forensic cases (Kerley, 1978).

In forensic science applications as well as those bioarchaeological, the purpose of the human osteologist is to help answer the following questions (White, 2000; Byers, 2004):

1. Is bone the material analyzed?
2. Are the remains human or non-human?
3. Are the remains modern?
4. What is the minimum number of individuals represented in the sample?
5. What was the *age* of the individual at death?
6. Are the remains of an adult? If so, what is the sex?
7. What was the height of the individual in life?
8. What is the ancestral affiliation of the individual?
9. Are any pathologies present on the remains?

The methods for the analysis of the biological profile (estimations of sex, age, stature, ancestry), as well as the rest of the questions posed above, have long been studied and described in a number of texts. Some of the most notable include those by Krogman

(1962), Stewart (1979), Isçan (1988), Buikstra et al. (1994), Bass (1995), Larsen (1997), and White (2000).

The estimation of age at death, an essential element in the creation of the biological profile, is the subject of the research contained herein. This is important because establishing the age at death in a human remains case can be a critical component in positive identification. By narrowing the age range, identification becomes more likely. Forensic Anthropology typically relies on standard morphological techniques for analyzing the age at death for both adults and subadults utilizing a multiplicity of methods that focus on the pubic symphysis (Todd, 1920; McKern and Stewart, 1957; Suchey and Katz, 1986; Brooks and Suchey, 1986), sternal rib ends (Isçan et al., 1984; Isçan et al., 1985), the auricular surface of the os coxa (Lovejoy et al., 1985), cranial suture closure (Meindl and Lovejoy, 1985, Mann et al., 1987) epiphyseal closure (Buikstra and Ubelaker, 1994), long bone length (Hoffman, 1979; Stewart, 1979), dental eruption and formation (Schour and Massler, 1941; Moores et al., 1963; Harris and McKee, 1990; Ubelaker, 1999), and dental wear (Murphy, 1959; Scott, 1979) among others.

Less pervasive throughout physical anthropology, forensic techniques of bone histology can also provide methodologies that estimate age at death (Martin et al, 1998; Robling and Stout, 2000). Histology is the microscopic study of plant and animal tissue (Ham, 1974). Not commonly used in forensics, histology has nevertheless been the topic of many innovative techniques of bone analyses for much of the 20th and 21st century. Bone histology can be used to determine if an unknown piece of material is bone, if it is human, or to identify the presence of pathologies. The estimation of age at death,

however, has been the focus of most histomorphological interest from the anthropological community of recent years, and multiple methods have been created and employed (Robling and Stout, 2000). In fact, the first published report of bone histology being used to estimate the age at death of a individual dates back almost 100 years to 1911 (Balthazard and Lebrun, 1911).

In bone histomorphometry, morphological components on a cellular level, such as bone cells and wall thickness, are measured (Eriksen, 1994; Kerley, 1965). Histomorphological research has led to prediction equations on the age at death of human individuals. These analyses typically depend on the measurements and/or tallies of variables such as osteons, osteon fragments and Haversian canals.

Generally, histological age estimation techniques utilize either the outer cortex or the entire bone cross-section. However, while histology can be an accurate method of age estimation, problems can often arise with individuals whose remains are not fully present, are fragmented, and/or are otherwise damaged. This results in an inability to perform the analyses. Additionally, some methods of histomorphology can be complicated. For example, specific analyses that focus on multiple characteristics require a higher level of training and experience to achieve success (Ahlqvist and Damsten, 1969; Stout and Gehlert, 1979).

In 1980, an article by Hauser et al. entitled "Identification using histomorphometry of the femur and tibia" (English translation) was published in the French periodical, Acta Medicinae Legalis et Socialis. This article focused on a new method of aging individuals through a histomorphological examination of the midshaft of the femur. Since then, the only mention of the Hauser method in published material has

been in the 2000 article by Robling and Stout which presented a basic summary of histological age estimation methods.

The Hauser method (1980) is made notable in part by its reported ease of use, as only one variable, the Haversian canal, is tallied. Additionally, and probably more important, the method utilizes two independent equations, one from the subperiosteal (outer cortex) and one from the endosteal (inner cortex) surface of bone. While it is always a plus to contribute an additional aging technique to histomorphology, the exciting possibility of the Hauser method lies in its endosteal equation. According to Hauser et al., the endosteal equation of bone is actually more accurate than that of the subperiosteum. As this author was unable to find any other histological aging methods that utilize the inner cortex of bone, the article by Hauser et al. provides a promising alternative. The ability to analyze only the endosteal surface of bone allows for age estimation when the subperiosteal surface of bone is damaged. If the endocortical method developed by Hauser et al. proves to be an accurate method, femora lacking an outer cortex will be able to be analyzed for both forensic and archaeological purposes.

The objective of this study is to analyze the Hauser method (1980) of age estimation in order to determine error rates, reproducibility, and comparability with a known histological method. The subperiosteal and endosteal equations will be treated as independent methods along with the average of the two age estimations provided by the equations. The better-known Kerley-Ubelaker method also utilizes the midshaft of the femur and will be included in this study for comparative purposes. Its variables focus only on the outer cortex of the bone cross-section. Since its original presentation in 1965, the Kerley technique has become a standard histological method for the determination of

age in the field of forensic anthropology (Kerley, 1965; Ahlqvist and Damsten, 1969; Stout and Gehlert, 1980; Robling and Stout, 2000). In 1978, Kerley and Ubelaker offered revisions of the microscopic field size for the method of determining age at death in human cortical bone.

In order to accomplish my goals, I will examine midshaft femoral cross-sections of 30 individuals of known ages on loan from Dr. Douglas Ubelaker at the National Museum of Natural History. Predictions of age from each of the Hauser et al. (1980) equations, as well as the average of the two estimations, will be compared to the actual ages at death of the individuals. I will also evaluate the Kerley-Ubelaker method (Kerley, 1965; Kerley and Ubelaker, 1978) with this sample. This will allow for a comparison not only among the Hauser equations, but also in contrast with that of the known Kerley-Ubelaker. The Hauser et al. method provides an additional option when severe fragmentation eliminates other histological methods, such as Kerley-Ubelaker. If the Hauser method, specifically the equation which utilizes the endocortical surface, proves to be an accurate method for estimating age, this opens the door for future research on the medullary cavity of bone.

Chapter 2: Histology

Bone Composition and Cell Types

As a type of connective tissue, each of the 206 bones in the human body can be studied microscopically. Bones are composed of three materials: water, mineral, and an organic matrix (Martin et al., 1998). While some water in calcified bone is free (flowing through pore space, not bound to minerals, capillaries, etc), a portion is bound to other molecules. Sixty-five percent of bone is made up of its mineral aspect. For example, Martin et al. (1998; p. 40) reports that "the mineralization of an *osteoid* (the organic portion of extracellular bone) displaces part of its water" resulting in a change of water content as new bone mineralizes. The mineral aspect of bone is mostly hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH_2)$]. This substance affects the solubility of bone mineral. The organic matrix of bone is largely composed of collagen, a structural protein. Collagen can "spontaneously organize itself into strong fibers" and gives bone its flexibility and tensile strength (Martin et al., 1998; p. 40).

Bone is a dynamic connective tissue that is in a constant state of change in reaction to its mechanical and physiological environment. There are four types of bone cells falling into the categories of those that resorb bone and those that form(ed) bone: osteoblasts (bone forming cells), osteoclasts (bone-resorbing cells), osteocytes (bone maintenance) and bone lining cells. Martin et al. (1998; p. 44) reports that osteoclasts resorb bone by eroding around the border of a cell "at a rate of tens of micrometers per day by first demineralizing the adjacent bone with acids and then dissolving its collagen with enzymes". Osteoblasts produce the organic portion of the bone matrix (osteoid). As an osteoid calcifies, an osteoblast may become surrounded by the intercellular organic

substance and is transformed into an osteocyte (Ham, 1998). Osteocytes are housed in lacunae (cavities) and communicate among themselves and osteoblasts through small tunnels (canaliculi). Similar to osteocytes, bone lining cells are dormant osteoblasts that "escaped being buried in newly formed bone and remained on the surface when bone formation ceased" (Martin et al., 1998; p. 48). These cells maintain a route of communication between themselves and osteocytes and are located on the endosteal and periosteal surfaces of bone. Bone lining cells, also converted osteoblasts, begin the remodeling of bone in response to mechanical stress, an extreme example of which is bone fracturing.

Bone Modeling and Remodeling

Primary and Secondary Bone

Compact bone can be divided into the two subgroups of primary (including circumferential lamellar bone and plexiform bone) and secondary bone (Martin et al., 1998). Primary bone is laid out onto the periosteal (outer) surface of bone during growth and development. Circumferential lamellar bone runs parallel to the surface of the bone and incorporates blood vessels "such that each is surrounded by several circular lamellae", which then form a primary osteon/Haversian system with a primary Haversian canal (through which blood vessels, lymph fibers and nerves pass) at its center (Martin et al., 1998; p. 37). To some extent, lamellar bone will be nonrandomly distributed through a cross-section reflecting an element of variation in the biomechanical loading between individuals (Robling, 1998).

Secondary bone occurs through remodeling, resulting in the resorption of existing bone with the creation of new lamellar bone (Martin et al. 1998). The cylindrical structures in secondary bone are labeled secondary osteons/Haversion systems containing about 16 lamellae surrounding the Haversian canal. Volkmann's canals, each conveying periosteal vessels, connect the Haversian canals to each other or to the marrow cavity and run transverse (perpendicular) to Haversian canals (Ham, 1998). As part of the aging process in some species (including humans), the majority of compact bone is replaced by secondary bone, which results in newly formed osteons as well as whole or partially absorbed older osteons. Secondary osteons/Haversian systems are circumscribed by discrete bundles of lamellar bone termed a cement line (Mulhern and Ubelaker, 2001). Trabecular bone also becomes secondary, though osteons are rarely produced in this environment since most often they do not fit inside of the individual spicules of trabeculae. Silberberg and Silberberg (1961) report that bone resorption is more dominant than deposition in bone older than 60 years at death. Examples of lamellar bone, Haversian canals, and Volkmann's canals can be seen in Appendix A, Figure 1.

Modeling and Remodeling

Modeling is the sculpting of bones during growth and development (Martin et al., 1998). More specifically, Ericksen et al. (1994; p. 2) states that bone modeling is "the process by which the overall shape of bone is changed in response to physiologic and/or mechanical change". This process results from the independent work of cells such as osteoclasts for bone resorption as well as osteoblasts for the formation of bone. This results in a change of the overall shape and/or size of a bone. Martin et al. reports that,

while the process of modeling is slow and prolonged, it becomes more quiescent as the skeleton reaches maturity (1998).

Remodeling is the life-long process by which bone is constantly renewed through the removal of older portions of bone (resorption) and replaced with that which is newly formed (mineralization) (Eriksen, 1994; Martin et al., 1998). This is usually in response to mechanical stressors (appearing for example, as fatigue damage) in order to "fine tune" the skeleton. That is to say, remodeling works to keep the skeleton in both a state of optimum mechanical competence (strength) and optimum physiologic competence (repair) (Frost, 1973). According to Frost (1985), the following are factors known to affect both osteon remodeling and the accumulated osteon populations:

- Age, chronologic
- Life span
- Sex
- Maturation, skeletal
- Species
- Hormones
- Electrolyte disorders
- Metabolic
- Genetic disorders
- Toxic agents
- Radiation damage
- Bone growth
- Bone remodeling patterns

The remodeling of a bone, according to Frost, is episodic, occurs throughout life (though there is a reduction as growth stops), and involves the sequential actions of osteoclasts (resorb bone) and osteoblasts (form new bone) working in basic multicellular units (BMUs) (1973). The endosteal surface of bone is characterized by higher activity through bone resorption than formation (Martin, 1998). It is this deficit in bone that "is

the reason for normal, age-related bone loss that occurs in men and women over the age of 30-35" (Martin, 1998; p. 66).

Each BMU is an instrument of bone remodeling that consists of about ten osteoclasts and several hundred osteoblasts that are activated by mechanical or chemical signals (Martin et al., 1998). In this process, bone structural units are created through the remodeling of cortical or trabecular bone. In a histological analysis, the extent to which the "bone remodeling unit . . . resorbs the preexisting cortical bone will determine the location of the reversal line and hence the area of the osteon" (Pfeiffer, 1998; p. 220). This can be viewed in Figure 2 in Appendix A. An accumulation of remodeling activity, as is associated with an increase in age, reflects in an increase in porosity and a decrease in cortical bone mass (Eriksen, 1994). Tersigni (2005; p. 18) summarizes the six stages involved the life cycle of an osteon:

1. Activation
   Differentiated cells are collected from a precursor cell population and are moved to the area in which the new osteon is to be formed.

2. Resorption
   Osteoclasts resorb bone by moving longitudinally through the bone at a rate of $40\mu m$/day. The BMU has a diameter of $200\mu m$ and a resorption area of a few hundred μm long.

3. Reversal
   Transition from osteoclast activity to osteoblast activity takes several days, and the reversal area is the lag space between the resorptive and refilling regions. The reversal line will eventually form the cement line. Process takes about 30 days.

4. Formation
   Osteoblasts appear at the periphery of the newly formed tunnel. They close approximately 1-$2\mu m$ per day. They leave a central passage (Haversian canal) for blood vessels to maintain the BMU, bone matrix and to carry calcium and phosphorus back and forth. This phase takes 3 months.

5. Mineralization

In this phase the osteoid is mineralized. There is a lag time of 10 days between the deposition of osteoid and the mineralization. Thus there is an osteoid front between the osteoblasts and the mineralized bone. Primary mineralization occurs within the first few days after mineralization starts. Secondary mineralization occurs as more minerals are added over the next several months.

6. Quiescence

After the BMUs have completed their process of remodeling the osteoclasts disappear and the osteoblasts become osteocytes and maintain the bone.

Thin Sections

When performing a basic histological analysis, bone is examined cross-sectionally. Although bone can be difficult to slice due to its calcification, several methods have been developed to create thin sections. Bone is routinely decalcified, dehydrated and embedded in paraffin before sectioning (Ham, 1998). Decalcified bone has had its original mineral composition dissolved away. This method is helpful in the preservation and thus dating of archaeological bone (e.g. 1,000 years B.P.) (Ham, 1988). However, the canaliculi are no longer observable, making it impossible to view bones' structural tissue on a dynamically changing level (Martin et al., 1998).

Two additional methods of producing thin sections of bone are available for more complex analyses. The first method of sectioning undecalcified bone involves the use of "special hard embedding media and special kinds of microtomes adapted for heavy work" (Ham, 1998; p. 382). Similar to paraffin embedding, the bones are immersed in liquid solutions, then polymerized and later cut into thin (less than ten micrometers or μm) or thick (100 micrometers or μm) sections (Martin et al., 1998). Bone that is burned, archaeologically relevant, or otherwise damaged can be dry, weathered and more likely to

crumble when sliced. By utilizing this thin sectioning technique, it is protected during the cutting process. This allows for a detailed analysis on a cellular level.

The older, second method, involves cross-sections termed ground bone sections, and has been popular since before the advent of plastics in the 1930s (Martin et al., 1998). The creation of ground bone sections involves the use of a fine saw to cut thin slices of calcified bone (Ham, 1998). This is followed by the grinding of each section until thin enough to transmit light. The thickness (50 or 100 micrometers for example) of the thin sections is varied to suit the needs of the specific research question. Again, cellular analysis is difficult with this method, but the canaliculi stand out, and the layered nature of bone and osteocytes are viewable as dark cavities. The latter method has been utilized in the sample studied for the analysis herein.

Chapter 3: Age Assessment

The aging of individuals is most easily determined through morphological methods. Once growth and development have ceased, however, estimating age at death can become more difficult. Furthermore, traditional morphological methods for determining the age can be useless in archaeological cases in which the "most useful indicators of age are often missing or obliterated in fragmented, eroded, or incomplete skeletons" (Kerley, 1965; p. 149). Unlike morphological methods of analysis, the microscopic evaluation of bone generally necessitates destruction of the bone itself. When determining the age of individuals older than 50 years at death, histological methods are required (Thompson, 1979). Creating thin sections generally involves cutting, grinding and polishing a section of bone followed by viewing it through polarized light. It is argued by Rösing et al. (2007) that while histology is more time consuming and destructive than morphological methods, it also yields more precise results.

Specific Examples of Methods

The tally of osteons or their fragments is commonly utilized in determining chronological age (Maat et al., 2006). Kerley (1965) developed age estimation equations involving similar tallies by using the femur, the tibia, and the fibula, though the femur is the only method that is explicitly explained. This was a landmark study for establishing a method of determining age histologically (Kerley, 1965; Ahlqvist and Damsten, 1969; Stout and Gehlert, 1980; Robling and Stout, 2000). Ahlqvist and Damsten (1969) compared the Kerley technique with a modified method they had devised that was less

laborious and required less skill. The results showed that both methods were valid, though Kerley's technique was more accurate. Later, these methods were again compared with similar results (Stout and Gehlert, 1980). It was noted that while Kerley's method was more accurate, there was far less interobserver error with that of Ahlqvist and Damsten. A later comparison by Stout and Stanley (1991) examined the accuracy of determining the percentage of a given area covered by osteons used by Ahlqvist and Damsten with the total count of osteons in the same given field as identified by Kerley. Results reported that osteon counts were a more accurate feature of analysis in histological age assessment than the percentage of cross-section covered.

Samson and Branigan (1987) utilized a contemporary sample from 58 individuals of European ancestry between the ages of 16 and 91 at death to develop an age estimation formula. They examined mean cortical thickness, mean Haversian canal diameter, and the number of Haversian canals per unit area of bone. Sexual dimorphism was found in these estimations. Female femoral cross-sections were not found to be accurate when analyzed. This formula was then applied to the femora of Iron Age and Saxon remains to estimate age at death. The authors reported very little variation when analyses were replicated. Similar to the Hauser method (1980), Haversian canals were a key aspect of the research and the method can be used on highly weathered or abraded bone.

Another aging technique involves taking a drilled core sample of bone four millimeters in diameter in order to minimize destruction (Thompson, 1979). The bone can then be left intact for subsequent analyses as well as to preserve the remains except for the hole drilled into it. This method includes the stereological quantification of secondary osteons and Haversian canals; the data are then applied to regression equations

that determine the age of the individual. In Thompson's analysis, the lower (femur and tibia) and upper (humerus) extremities were examined and yielded positive results, although estimations of the lower extremities were identified as marginally more accurate. Thompson (1981) continued the original study by core sampling a larger sample (54 individuals) with known ages. The humeral method was determined to be too inaccurate for identification applications. The femoral and tibial results, although they exhibited general accuracy, indicated that the analysis was slightly more accurate for females than males. Additionally, this method was compared among "white", "black" and "Eskimo" individuals and indicated a greater accuracy in the determination of age for skeletons identified as "white" (Thompson, 1981).

Stout et al. (1994), in contrast to earlier methods, developed the use of the sternal end of the fourth rib in place of long bone analysis. The authors disputed Thompson's (1979; 1981) and others' methods of extracting small portions of bone, such as a core, by stating that these are not complete representatives of bone. Stout et al. (1994) examined intact osteon density, fragmentary osteon density, and total osteon population density. Given the small sample size of the bone, this method is especially intriguing since a cross section of the entire bone is used. The use of a complete cross-section results in a more comprehensive assessment of the histology of the bone than with methods such as Kerley (1965) or Thompson (1979; 1981). Additionally, use of the fourth rib leaves the long bones intact for other osteometric analyses. The sternal end of the rib is also easily accessible anatomically, and the method requires only a minimal amount of bone. This allows a morphological analysis of the sternal end of the rib to still be possible in the same sample.

Analyzed bones do not need to be of recent origin to be useful for age determination. Stout (1986) utilized a prediction equation of age at death using a rib sample to identify unknown skeletal remains over 400 years old. The age predicted was consistent with ages reported through historical documentation. A study by Ericksen (1997) focused specifically on the use of histology in bioarchaeological settings. The sample included 122 Preceramic adult skeletons excavated from a cemetery in Chile dating to approximately 3300 to 3000 B.P. Previous research on the remains had developed and assigned morphological ages at death. The anterior cortex from the midshaft of the femur was then extracted for histological analysis. Results suggested that the use of histological analyses for the determination of historical age has tended to over-age individuals (Ericksen, 1997). Ericksen hypothesized that this could be due to different distributions of lifeways (such as differential environments, diets and/or physical activities) between past and contemporary populations. Another factor in the over-aging of individuals could be the application of equations that were initially determined based on samples of older (e.g. mean = 50s) individuals and then applied to groups that died at younger ages (e.g. mean = 30s). Additionally, caution is emphasized in the analysis of skeletal microstructures as the "exfoliation of peripheral unremodeled bone lamellae can cause over-aging as deeper, more remodeled areas come to lie nearer the periosteal surface" (Ericksen, 1997; p. 65).

Overall, there is a variety of histological methods to determine age at death utilizing bones from throughout the human skeleton. The most accurate methods have incorporated either the rib or the femur as the location for analysis. Accurate methods of age estimation for the microscopic analysis of bone have been available for over 40

years, but its utility in the forensic world had been surprisingly small (Maat et al., 2006). With a more complete comparative understanding of aging techniques on past and present populations, modifications of the methods of age assessment can more accurately compensate for differences in bone structure. Given the difficulty of the age assessment however, it is ultimately preferable to consider several indicators of age simultaneously (Rösing et al., 2007).

Factors Affecting Age Estimation

Bones Utilized in Analysis

When assessing age by analyzing cortical remodeling, the sampling location is key (Pfeiffer et al., 1995). Methods for the aging of human remains have involved a variety of bones including the occipital (Cool et al., 1995), second metacarpal (Kimura, 1992), mandible (Singh and Gunberg, 1970; Drusini and Businaro, 1990), fibula (Kerley, 1965; Kerley and Ubelaker, 1978), ulna (Thompson, 1979), femur (Kerley, 1965; Ahlqvist and Damsten, 1969; Kerley and Ubelaker, 1978; Thompson, 1979; Eriksen, 1991; Hauser et al., 1980; Samson and Branigan, 1987;), tibia (Balthazard and Lebrun, 1911; Kerley, 1965; Kerley and Ubelaker, 1978; Hauser et al., 1980), humerus (Iwamoto et al., 1978; Thompson, 1979; Yoshino et al., 1994), sixth rib (Stout and Paine, 1992), fourth rib (Stout et al., 1996), and clavicle (Stout et al., 1996). The femoral midshaft, however, remains the most utilized area of the body (Chan et al., 2007).

Ribs are considered by some to be the ideal site for histological analysis because an entire cross-section, rather than only a portion of the bone, can be analyzed, as in the case of long bones (Crowder and Rosella, 2007). Additionally, ribs are noted to reflect

the minimal presence of biomechanical variation. Robling (1998) suggested the creation of an age-at-death formula that takes mechanical loading into account. The applications of these formulae have limitations, however, because ribs, having thin cortices, are less resilient to taphonomic processes than long bones. Additionally, various aging techniques rely on the specific areas (such as the middle third) of a particular rib (such as the sixth rib). When skeletal remains are discovered with damaged or incomplete rib sections, the positive identification of the specific ribs can prove impossible. Crowder and Rosella (2007) have documented intracostal variation in rib histomorphometry. They reported a high level of variable osteon counts among ribs in the same individual. Therefore, the use of the rib for age determination is only a good choice when the specific rib is identifiable and the cross-section is whole.

Other histological age estimation methods include analyses based on human teeth and infants. Fitzgerald and Rose (2000) interpreted markers of internal microstructural growth of teeth to estimate age. These markers were noted to be a valid area for age estimation for situations in which the teeth are complete and other age estimation methods are unable to be used. There have also been studies assessing the long bones of infants and children for histological subadult aging (Jowsey, 1960; Kerley, 1965). Unfortunately, subadult skeletons are thought to be too complex for analysis due to the stacking of bone modeling and remodeling that makes analyses of cellular based structures inconclusive (Stout, 1992).

Physical Activity

Pfeiffer (2000, p. 298) reports that "histological patterns of human cortical bone appear to be somewhat distinctive among mammals, and they appear to vary temporally

and spatially". The estimation of an individual's age can be biased when the level of physical activity of the general population analyzed is unknown. Indeed, when the population upon which a particular method is based is unknown, the level of error between the groups can be extremely high. If an age estimation method was based on a highly active group of individuals, and this technique was then applied to one sedentary individual, the results could be significantly biased. One clear example of this was provided by Ubelaker (1974) who examined the ages at death of individuals from two Late Woodland ossuaries in Maryland. He compared the gross aspects of the pubis with the histological aging method developed by Ahlqvist and Damsten (1969). This method was based on a modern Finnish population. When it was used to estimate the age of Ubelaker's sample, the results deviated highly from those obtained by pubic analysis (1974). Ubelaker offers reasoning that this discrepancy may be due to unequal activity levels.

Physical activity can significantly increase the error rate of an age estimation. An animal study by Lieberman (1997) demonstrated that an increased level of mechanical loading corresponds to an amplification of intracortical bone remodeling. Chan et al. (2007) caution against the use of the posterior, lateral, and medial locales due to significant circumferential variation caused by biomechanical loading and unloading. Hauser et al. (1980) specifically suggest that the posterior aspect of the periosteal surface of bone not be included in analyses because of the presence of the linea aspera, a strong muscle attachment site on the femur.

Location on Bone for Analysis

The midshaft is the most common area for analysis in long bones such as the femur. Chan et al. (2007, p. 81) reports that "cross-sections evaluated from the proximal region of the femoral cortex will show a negative age estimation bias" and "those taken from the distal diaphysis will show a positive bias" in comparison with the standard anterior midshaft age estimates. Tersigni (2005) reports significant inter- and intra-sectional variation as related to age estimates by the Kerley (1965) method. Tersigni (2005) determined that the middle two inches (approximately) of midshaft femoral bone are acceptable for use in cross-sectional age analysis as this area is indicative of the lowest error rates along its length.

Sex Variation

The idea of variation between the sexes has been present at least since the original presentation of the Kerley (1965) method of age estimation. Kerley (1965), Stout and Paine (1992), and Stout et al. (1994) have all reported no difference in age estimation based on the sex of the individual. Others have cited Kerley's (1965) conclusion as a reason for disregarding the sex of individuals altogether in their development of age estimation methods (Ahlqvist and Damsten, 1969). On the other hand, Singh and Gunberg (1970), Samson and Branigan (1987), and Yoshino et al. (1994) all only examined male specimens, leaving the question of variation between the sexes unaddressed.

Thompson (1979; 1981) and Ericksen (1991) decided to face this question head-on by creating separate equations for males and females. Thompson (1981) reported more accurate results in female estimations than in males. Thompson did not, however,

check accuracy of the sex equations when the results were pooled. Ericksen (1991) also found that separate sex equations were more accurate, noting that females accumulate intact osteons into their sixties while males can accumulate osteons into the one hundredth year of life. An uneven distribution in the size of osteons between males and females is an additional consideration relating to sexual variation. In a recent study by Pfeiffer (1998), however, no sex differentiation in osteon size of the femoral midshaft and sternal 1/3 of the sixth rib were noted among samples from an English population of the 18th century, Canadians of the 19th century and South Africans of the 20th century.

Although differences in the accuracy of age assessments between sexes have been reported to be negligible, females have been found to lose cortical thickness at a higher rate than males (Walker, 1982). A negative correlation was also identified between cortical thickness and age. Research by Walker (1982) recorded the greatest loss of cortex in the long bones of females after the fifth decade. However, Robling and Stout (1998) reported a rapid increase in the osteon density of females immediately after the onset of menopause. These factors may affect the estimation of age of women older than 50 years at death. However, following Ahlqvist and Damstens' study (1969), I will not take sex into account in these analyses. Additionally, neither Kerley-Ubelaker (1978) nor Hauser et al. (1980) separated their method equations by sex.

Population Variation

Ancestral variation is a notable factor in age determination. Thompson and Gunness-Heyy (1981) asserted that Eskimos appear to have a higher rate of bone turnover than European Americans. These researchers applied Thompson's (1979) histological method to 19th century Eskimo femora and obtained poor age estimates.

Unspecified human cadavers were the original population utilized in order to create the technique achieved by Thompson (1979). Nothing is mentioned as to ancestral derivations and variations among these specimens. Thompson and Gunness-Heyy (1981) concluded that population-specific equations are necessary to account for genetic differences in the remodeling of bone. Similarly, Ericksen and Stix (1991) used a 19th century American sample deriving from Philadelphia to test a technique created by Ericksen based on a sample from the Dominican Republic. The method was determined to be successful. However, the age of those falling outside the bounds of the method's standard error were generally over-estimated.

Kerley's (1965) method, and its later revision by Kerley and Ubelaker (1978), was originally based on a sample of 126 American military personnel said to be predominantly white and male. Both the original Kerley method and its revision have also been applied to samples of different ancestries. Fangwu (1983) tested a sample of modern Chinese femora (n=35) using the original Kerley (1965) method. It was found that approximately 42% of the estimated ages were within ± 5 years and 67% were within ± 10 years of actual age at death. Thirty-three percent of age estimations had error rates of greater than ± 10 years.

Additionally, Ubelaker (1981) tested the revised Kerley-Ubelaker (1978) method on a sample of 114 femora from the Dominican Republic. A sub-sample of these specimens was analyzed in the research for the present thesis. Ubelaker's results indicated that 42% of the age estimations were within ± 5 years of the actual age at death, while 62% were within ± 10 years. Although the rate of error is higher than that established by the Kerley method (based mainly on American soldiers), it is not high

enough to preclude the applicability of the method to a demographic reconstruction. The large size of the sample analyzed tends to normalize the data.

Destruction of Bone

Another problem in age assessment utilizing an entire cross-section of the mid-diaphyseal femur is that the act of cutting the cross-section is destructive. This destruction can preclude analyses of the biological profile (i.e. stature, ancestry) as well as biomechanical stresses. As Robling (1998; p.163) suggests, "histologic estimates of age at death that rely on the microstructure of dynamically loaded bones (e.g., femur, tibia) are affected by the mechanical usage of that limb". Alternatives to an entire cross-section of the femur being utilized include some comparably accurate age estimation methods (Thompson, 1979; Iwaniec et al., 1998; Stout, 1994) that attempt to preserve the integrity of the bone and/or rely on areas less subject to biomechanical stressors. These methods accomplish this by drilling a core of bone (Thompson, 1979), disturbing only a section of the bone (anterior femur) (Iwaniec et al., 1998), or the use of an entire cross-section (rib) (Stout, 1994).

Intra-Observer Error

Variation in intra- and inter-observer analysis for histological techniques through the comparison of differing identifying features (secondary osteons, Haversian canals, osteon fragments) was studied by Lynnerup et al. (1998). The results revealed that the only feature not resulting in large inter- or intra-observer error rates in the identification of features was secondary osteons. Some difficulty was noted in positive identification of Haversian canals through its possible overlapping (through the wide limits of identification) with osteon fragments. The authors suggested that osteon fragments

should not be included in any analysis. Though unstated, it is assumed that Haversian canals should also be excluded.

There is a key discrepancy in the research of Lynnerup et al. (1998). It did not analyze the identification of Haversian canals as the sole feature of analysis. It is possible that when a researcher is identifying only Haversian canals, the lines between the canals and the osteon fragments are less blurred. In the independent identification of Haversian canals, it is the thought of this researcher that, through intra-observer analysis, the Haversian canals will not suffer high rates of error.

Chapter 4: Additional Histomorphometric Applications

Forensic anthropology and bioarchaeology have naturally overlapping goals in histological analyses. This is true beyond histomorphological age estimations. When bones of an individual are discovered, the same questions are asked whether they died four weeks or four hundred years ago (Who were they? How did they end up here?). As such, many techniques are applicable to both forensic and archaeological applications.

Bioarchaeological Applications

Bone is often the only type of human tissue to be preserved in archaeological settings. As such, morphological and metric analyses of the skeleton are often used to infer the lifeways of past populations. Both Robling (1998) and Pfeiffer (2000) reported that histology can be used to deduce activity levels in human skeletal remains. Problems arise when poor bone preservation or extreme fragmentation render these methods insufficient. For example, when skeletons are excavated in archaeological situations, bones can often suffer from post-depositional crushing (Boddington, 1987).

Bone histology has developed as a legitimate alternative to morphological and metric methods. This is due to a recent increase in research in the microscopic analysis of past populations. For example, Pfeiffer (1998) has used histology to compare Late Pleistocene human samples with those of nineteenth century cadavers in order to analyze the diversity between the populations through the histomorphometric identifications of sex, age, and population/environment. She found that while these patterns appear to be somewhat distinctive among mammals, they also vary temporally and spatially.

The most common argument against the use of histology in the analysis of archaeological bone has been that alterations over time result in the obliteration of the microstructure of bone (Jackes et al., 2001). These modifications, examined histologically, can be separated into four categories according to Cipollaro et al. (1999; p. 3):

1. Good Preservation
   The microstructure of bone is "completely preserved and the different structural elements (osteons, osteon fragments, and interstitial lamellar systems) were clearly distinguishable."

2. Intermediate Preservation
   There is partial alteration of the bone microstructure.

3. Poor Preservation
   The majority of the overall bone microstructure is altered.

4. Very Poor Preservation
   The overall microstructure is altered.

Differential bone degradation seems to be caused, at least in part, by the original deposition of bones into humid and/or variable (non-stable) environments resulting in microbial breakdown (Jackes et al., 2001). Additional decomposition factors suggested by Child (1995) include burial temperature, alkaline and acidic soils, microbial interactions, non-microbial inhibitory substances, extremes of temperature, and enclosed environments. Interestingly, however, the degradation of bone does not "show a linear relationship with time" (Jans et al., 2002; p. 349). A study by Bell et al. (1996), who analyzed the histological cross-sections of eleven individuals, reports that early postmortem alteration to skeletal tissues can appear as soon as three months after death. In contrast, a specimen one year after death can remain in excellent microscopic cellular condition.

Jackes (2000) suggests caution in employing histological analysis when analyzing archaeological bone. All individuals should not be utilized as the process is inherently destructive. Instead, it is suggested to give an overall consideration to what could be done to produce the quickest, most accurate and most economical result. While macroscopically bone may appear healthy, the outer portion of bone can possibly be unusable for histological analysis on a microscopic level (Jackes, 1992).

Garland (1987) discussed the relationship between burial environment and histological appearance in an article researching the histological structure of fossil bone. It was found that bones which have had their histological structure destroyed or altered may not be used for the purpose of biological aging by several of the current histological methods. However, Haversian canals were reported as easier to identify than osteons and osteon fragments in older disinterred bone. Specimens examined by Garland reflect the possible positive use of Haversian canals for a burial dated to the first century A.D.

While these factors can be an impediment in the analysis of archaeological samples, histology can still be useful and, in some cases, completely applicable. For example, Cipollaro et al. (1999) used histology alongside DNA amplification in the study of remains almost 2000 years of age. While some of the bones were completely degenerated, others in the analysis exhibited microstructures that were indistinguishable from fresh bone, possessing osteons, osteon fragments, and interstitial lamellar systems.

Within archaeological contexts, bone histology is a useful path for the analysis of human versus nonhuman remains and the age assessments of individuals. This is especially applicable after the cessation of growth and development. This field

additionally contributes to the study of diet, disease and bone degeneration in present and past populations.

Histology can be a useful tool in the determination of the preservation of bone microstructure in heritage management (Jans et al., 2002). Excavation is a destructive process no matter how careful the archaeologist. When a site is disrupted, it can never be restored to its previous state, and material remains can be destroyed. It is because of these factors that many archaeologists in heritage management currently prefer to preserve remains in the environment in which they had survived since deposition. In order to make in situ preservation a reasonable alternative to excavation, however, "more knowledge is required about degradation mechanisms of archaeological materials" and their influencing factors (Jans et al., 2002; p. 344). Histology can contribute significantly to these preservation issues. The destruction of bone over time, touted as a weakness in the use of histology in bioarchaeological research, has been made a strength in this section of analysis.

The microscopic study of bone diagenesis can contribute more to interpretations of the state of bone preservation than simple macroscopic evaluations. Thin sections of bone must meet several criteria for histology to be a useful technique for archaeological management research. These conditions include an overall "impression of the preservation status of the bone" and demonstrate relatively small changes therein (Jans et al., 2002; p. 350). With histology, degenerative changes can "be localized within the bone, giving a more detailed picture of diagenesis within the bone" (Jans et al., 2002; p. 350). It is important to produce long-lasting, high quality thin sections when analyzing

bone for this purpose (Garland, 1989). These sections can provide avenues for research in the future as new techniques become available.

Human/Nonhuman Identification

The most basic question asked upon discovering bone in archaeological contexts is, "Is it human?". This can generally be answered by looking at the morphology of bone but can become far more difficult in situations of severe fragmentation. Microscopically, animal bone can be readily differentiated from that of human, as they possess different cortical and cellular patterning (Gray, 1941). A number of characteristics can be used to help in this differentiation. Gray (1941) used the number of canaliculi that arise from a lacuna in femora and parietals to differentiate human from nonhuman bone.

Plexiform bone is a main method of differentiation between human and nonhuman bone. It is rarely found in humans and is characterized by a mixture of lamellar and woven bone which often appears as a "brick wall" as it contains rectilinear spaces that are residuals of past vascularity (Martin et al., 1998; Mulhern and Ubelaker, 2001). Plexiform bone is typical in large animals that have rapid growth rates, such as cows (Martin et al., 1998).

While plexiform bone is a common distinguishing feature between human and nonhuman bone, other methods are available. A study by Mulhern and Ubelaker looked at the differences in osteon banding, defined as at least 5 distinctive rows of primary and/or secondary osteons (2001). They determined that osteon banding not only occurs more often in nonhuman bone, but that the bands that appear in human bone are far less organized. For example, small animals such as rats have fine-fibered bone in their

cortices which presents as randomly arranged collagen fibers that are smaller and more closely placed than woven bone (Martin et al., 1998).

Cattaneo et al. (1999) looked at possibilities surrounding the differentiation of human from nonhuman bone in the case of badly burned bone fragments. They conducted a study comparing histological, immunological, and DNA techniques on burnt bone. This was meant to be an analysis using the "worst case scenario" with temperatures between 800-1200°C in order to simulate the environment of a house or car fire. Histologically, bones were analyzed through both the morphology of microscopic thin sections and a quantitative, metric analysis. For the metric analysis an "algorithm for histological species identification from fragments of the type of bones commonly found among burnt remains, as for example in the present forensic cases" was developed (p. 188). Though simulated in severe temperature, the cellular matrix of the bone remained relatively unchanged and was readily identifiable. This indicates that histological analysis can be comparable to immunology of the DNA analysis of bone (at least in reference to human versus nonhuman bone). It is also indicative that histology is successful, at least to a certain degree, in analyzing burnt bone.

Taking into account osteon shrinkage that occurs with burning, the analysis reported by Cattaneo et al. (1999) was based on both metric and morphological thin-section histology. The histological reference data for burnt bone included three variables: the diameter, perimeter and area of the present osteons and Haversian canals. This information was then utilized to create an equation for the metric determination between human and nonhuman bone. Conversely, the biomolecular analysis involved a test for the presence of human albumin and DNA amplification. The authors concluded that the

quantitative histology conducted was far more successful (79% correct) than biomolecular techniques (9.5% correct) in identifying the human from nonhuman origin (Cattaneo at al., 1999).

Skeletal Pathologies

Archaeological histology samples can present information on the health of past populations. Some skeletal pathologies are specific enough to differentiate from others microscopically (Lovell, 2000). Paine and Brenton's 2004 study focused on the analysis and diagnosis of paleopathology through histology. Specifically, *pellagra,* a niacin deficiency disease, was examined. The sample included 27 individuals from the Raymond Dart collection in South Africa who were known to have died either from pellagra or general malnutrition. The authors examined the relationships among Haversian canals, lacunae, and secondary osteons of the rib samples. Distinct differences were found between the two groups. Pellagra was shown to exhibit extremely thin cortical bone and large Haversian canals in all samples. Other common characteristics for this disease process include type II and double zonal secondary osteons, and Howship's (resorptive) lacunae. This study adds to the "understanding of the diet and health of maize-dependant populations of both past and present" (Paine and Brenton, 2004, p. 156).

A similar study, presented by Cook et al., focused on the "histomorphometric study of thin femoral head sections of a skeletal sample from the Dakhleh Oasis, Egypt, dated from circa 36 B.C. to 400 A.D." (1988, p. 23). The parameters measured include the mean wall thickness, trabecular bone volume, surface volume, mean trabecular

diameter, and total resorption surface. A high total resorption surface (12.31%) was noted in relation to hyperparathyroidism. The results highlight histology's use as an investigative tool in the detection of disease, specifically hyperparathyroidism in archaeological samples dating at least 1,600 to 2,000 years B.P. (Cook et al., 1988).

Chapter 5: Questions and Hypotheses

The main goal of this study is to test the Hauser et al. method of histomorphological age at death estimation. The establishment of error rates, reproducibility and the overall accuracy of the Hauser et al. technique are the main objectives in this project. Further, it is the intention of this study to examine the endocortical equation of Hauser et al. specifically as an age estimator. A direct comparison of the Hauser et al. method to that of Kerley-Ubelaker will showcase the applicability of this method to one already well established. From this point on, the age estimated by the average of the Kerley-Ubelaker osteon and osteon fragment ages will be KUA; that of the subperiosteal Hauser et al. equation will be HPA; that of the endosteal Hauser et al. equation will be HEA, and the estimated average of both Hauser et al. age equations (subperiosteal and endosteal) will be HAA. This study seeks to answer a multiplicity of questions:

Research Question 1

What are the correlations between real ages from the Ubelaker slide sample and the predicted ages for each histological method (KUA, HPA, HEA, and HAA)? Does there appear to be an overall difference?

If the estimations of age based on each method are perfect, the resulting correlation will be 1.0. The overall correlation between the age estimation of the Kerley-Ubelaker method (KUA) and the chronological age should be close to 1.0; i.e. a significant overall difference between them will not be indicated. Also, there will not be an overall difference in the accuracy of the age estimation of the Hauser et al. periosteal

equation (HPA), the endosteal equation (HEA), and the average of the periosteal and endosteal Hauser et al. equations (HAA) when compared with the Ubelaker slide sample.

Research Question 2

How does the real age (RA) from the Ubelaker slide sample compare with each method's (KUA, HPA, HEA, and HAA) estimated age? If present, what is the directionality of these techniques?

Question 2a: Will there be a statistically significant difference in the overall age estimations by: KUA, HPA, HEA, and HAA?

Question 2a: Hypotheses

H_{o1a}: No statistical difference exists between the mean numbers of years estimated from real for KUA.

H_{a1a}: There is a statistical difference in the mean numbers of years estimated from real for KUA.

H_{o2a}: No statistical difference exists between the mean numbers of years estimated from real for HPA.

H_{a2a}: There is a statistical difference in the mean numbers of years estimated from real for HPA.

H_{o3a}: No statistical difference exists between the mean numbers of years estimated from real for HEA.

H_{a3a}: There is a statistical difference in the mean numbers of years estimated from real for HEA.

H_{o4a}: No statistical difference exists between the mean numbers of years estimated from real for HAA.

H_{a4a}: There is a statistical difference in the mean numbers of years estimated from real for HAA.

Question 2a: Expected Outcome

It is unknown if there will be a statistically significant difference in histological age estimation generated in the overall usage of the Kerley-Ubelaker as well as the Hauser-P, Hauser-E, and Hauser-Avg (HPA, HEA, HAA) Hauser et al. equations.

Question 2b: Is there a directionality of each method of age estimation (KUA, HPA, HEA, and HAA) from the actual ages of individuals at death? If there is a directionality, is it significant? Do the individual methods underestimate or overestimate the actual age at death of individuals?

The expected outcome of the overall presence of directionality is unknown for each of the techniques analyzed (KUA, HPA, HEA, HAA). According to Ubelaker (1981), the Kerley-Ubelaker method should prove to both underestimate and overestimate age. The Hauser-P should prove to overestimate age and Hauser-E should underestimate age based on preliminary research by the author. Similarly, the averaged results of Hauser-P and Hauser-E (HAA) should prove to generally cancel out underestimations and overestimations.

Research Question 3

How do the histological aging techniques (KUA, HPA, HEA, and HAA) compare among each other?

Question 3a: What are the correlations among KUA, HPA, HEA, and HAA? Does there appear to be a difference?

The intercorrelation rates among the four methods when analyzed with the Ubelaker slide sample should be close to 1.0 as all methods are expected to have similarly high correlation rates when compared to the actual ages of individuals.

Question 3b: Is there a statistically significant difference in the estimations of age among KUA, HPA, HEA, and HAA?

Research Question 3b: Hypotheses

H_{o1}: There will be no statistical difference in the mean difference between KUA and HPA.

H_{a1}: There will be a statistical difference in the mean difference between KUA and HPA.

H_{o2}: There will be no statistical difference in the mean difference between KUA and HEA.

H_{a2}: There will be a statistical difference in the mean difference between KUA and HEA.

H_{o3}: There will be no statistical difference in the mean difference between KUA and HAA.

H_{a3}: There will be a statistical difference in the mean difference between KUA and HAA.

H_{o4}: There will be no statistical difference in the mean difference between HPA and HEA.

H_{a4}: There will be a statistical difference in the mean difference between HPA and HEA.

H_{o5}: There will be no statistical difference in the mean difference between HPA and HAA.

H_{a5}: There will be a statistical difference in the mean difference between HPA and HAA.

H_{o6}: There will be no statistical difference in the mean difference between HEA and HAA.

H_{a6}: There will be a statistical difference in the mean difference between HEA and HAA.

Question 3b: Expected Outcomes

The statistical difference among each of the histological aging techniques (KUA, HPA, HEA, and HAA) is unknown.

Research Question 4

What will the intra-observer error correlation rate be in the analysis of the age estimation equations (KUA, HPA, HEA, and HAA)?

If no intra-observer error is found, then the r-value of the correlation will be 1.0. However, if the rate of intra-observer is high, the correlation will be closer to 0.0. The correlation rate of intra-observer error in this study is unknown.

Chapter 6: Materials and Methods

Sample

The sample in this analysis consists of slides on loan from Dr. Douglas Ubelaker at the National Museum of Natural History in Washington, DC. The sample was originally collected in the Dominican Republic in 1974. It encompassed the known age and sex of a total of 158 skeletons representing underprivileged individuals "removed from local cemeteries as a solution to space problems" and were "socially classified as Black or mulatto" (Ubelaker, 1981; p. 183). These individuals were reported to be mainly laborers.

Thin sectioned in 1975, ground sections of 114 individuals were successfully created from the midshaft cross section of the femur. Forty-four of the sectioned samples were rejected based on:

> "(1) displayed loss of bone from the periosteal surface due to erosion in the soil or fragmentation during the preparation process, (2) displayed excessive infiltration of soil or organic debris within the periosteal margin of the cortex, (3) were from cross sections which displayed evidence of pathological disturbance, and (4) were from skeletons of questionable age at death" (Ubelaker, 1981; p. 184).

Unidentified skeletal remains include a wide spectrum of individuals (sex, ancestry, height). This diversity also carries over into age at death and, as such, the sample for this study was chosen to reflect this. From the Ubelaker sample, 30 femoral cross sections have been chosen (N=30), representing a broad age range of nine to 82 years of age at death. As the glue utilized for some of the original mountings had begun to deteriorate, the decision of which samples to choose from the original 114 was based on the age of individuals at death and the overall quality of the Dominican Republic slides. The sex of

each individual was recorded, although it is not expected to be a significant variable when analyzing age histomorphologically (Ubelaker, 1981).

Methods

This research was conducted in the Forensic Anthropology Laboratory in the Department of Anthropology at Michigan State University. The slides were placed under a Leica MZ 12 stereomicroscope attached to a Dell Dimensions 4550 computer (Appendix A, Figure 3). A stereomicroscope is the compilation of two microscopes focusing on one point from different angles allowing for a three-dimensional and correctly upright and lateral view of an image (neither upside down nor backwards). The computer program, Sigma Scan Pro 5.0, was then utilized in acquiring the image of each slide. Each image was captured at 10x with a correction factor multiplied by each of the tally totals for Kerley-Ubelaker (osteons, osteon fragments, non-Haversian canals, and percentage present lamellar bone) as well as Hauser et al. (endosteal, periosteal). Described below, the program also allowed for a tallying of specific points of interest for each equation.

Captured images were used to analyze the Kerley-Ubelaker method (KUA), the subperiosteal equation of the Hauser method (HPA), the endosteal equation of the Hauser method (HEA), and the average of the Hauser equations (HAA). Each image was then saved on the computer as the plain image (no field size) by way of a Sony camera adapter CMA-D2 and viewed on a Sony Trinitron (Appendix A, Figure 3). Each captured image was rectangular in shape (1.30 x 1.00 mm). As the fields for both Kerley and Hauser are circular, a correction factor was then multiplied by the final tally for each individual in

each feature analyzed. My analysis was accomplished through the use of both the captured computer image and the microscope. Each method was performed twice for the 30 individual samples. These actions were performed in order to help ensure minimal intra-observer error as well as provide easy reference of the analyses.

As Stout and Gehlert have stated, "the histomorphometrics of bones from different individuals can be quite similar, especially if the individuals are similar in age" (1979; p. 803). However, through the combination of age analysis due to cortical remodeling, the osteon density of bone, reflections of biomechanical stressors due to physical activity, and cortical rift patterns from altered biomechanical forces can result in the individualization and positive identification of bone. The above authors accede, however, that bone histomorphology requires a great deal of experience (Stout and Gehlert, 1979; p. 804). As such, it should be noted that this author does not possess extensive experience in histological age analysis and is partially self-taught.

Kerley-Ubelaker

The 1978 Kerley-Ubelaker revision of the original Kerley method (1965) was utilized in this study. The sample included in this study is a subset (n=30) of the one in which Ubelaker identified the error rate in examining the Kerley-Ubelaker method (N=114 femora) averaging 10.43 years (1981). This analysis, however, did not specify the variable used for the age estimations (see possibilities below). The author will take into account past analyses and note it in the discussion section.

Each image was captured with Sigma Scan Pro 5.0 through the Leica MZ 12 stereomicroscope at 100x. The anterior, posterior, medial, and lateral aspects of each

slide of midshaft femoral bone were assessed. Each area was examined within the constraints of the field size for osteons (at least 80% complete), osteon fragments, and lamellar bone and primary osteons. Osteons, osteon fragments, and primary osteons were counted (number present tallied) and the percentage of present lamellar bone estimated and placed into separate equations. According to a 1982 study by Stout and Gehlert based on individuals aged 13-51 years, non-Haversian canals significantly underestimate the actual age of individuals while osteon and osteon fragments will hold greater weight in the final analysis. The number of present non-Haversian canals was collected but did not receive weight in the overall age analyses. This was also true for the percentage present of lamellar bone consistent with analyses by Stout and Gehlert (1980; 1982). It was decided that the percentage would not be included due to the variability inherent in collecting the data (estimating percentages). Additionally, Kerley-Ubelaker identify both non-Haversian canals and percentage of present lamellar bone as possessing the highest level of error (1978). As such, the regression equations utilized for statistical analysis of the Kerley-Ubelaker method only used those of osteons and osteon fragments.

Table 1. Regression equations for Kerley-Ubelaker (1978) age at death estimation
(Y = Age in years; X = Osteon or Osteon fragment count)

Variable	Formula
Osteon Age (OA)	$Y = 2.278 + 0.187X + 0.00226X^2$
Fragments Age (FA)	$Y = 5.241 + 0.509X + 0.017X^2 - 0.00015X^3$
Osteon/Osteon Fragment Average (KUA)	$Y = \frac{(2.278 + 0.187X + 0.00226X^2) + (5.241 + 0.509X + 0.017X^2 - 0.00015X^3)}{2}$

The field size directed by Kerley-Ubelaker (1978) is circular with an area of 2.06 mm^2 (diameter of 1.62 mm). The area of the stereomicroscope field was rectangular with an area of 1.30 mm^2 (length by width of 1.30 mm x 1.00 mm). The correction factor was then determined to be 2.06 $mm^2 \div 1.30\ mm^2 = 1.585$. The total tally number of each feature was then multiplied by 1.585 and entered into the specific regression equation (see Table 1). The age estimations for each regression equation were then averaged to obtain a single age for the individual.

Hauser et al.

For the 1980 Hauser et al. method, the area of the circular field was decreased to 1.056mm^2 (diameter 1.16 mm). The rectangular area of the stereomicroscope field of 1.30 mm^2 (length by width of 1.30 mm x 1.00 mm) was then utilized with the Hauser field size to determine the correction factor (CF). The equation involved was 1.056 $mm^2 \div 1.30\ mm^2 = .812$. The total tally for each of the two equations provided by Hauser et al. was then multiplied by the CF. The resulting corrected tally was entered into each of the regression equations (see Table 2). Two areas of each bone (subperiosteal and endosteal) were utilized for these separate equations. As such, they were treated as separate methods and are detailed below. The Hauser et al. technique of histological aging utilizes three aspects for each of the equations of the femoral thin section with an additional three sub areas for each of the aspects.

SubPeriosteal Surface

Hauser et al. (1980) decided not to use the posterior aspect of the femur for their subperiosteal equation. This, they state, is due to the large variations in the size and

number of osteons for the posterior region as the linea aspera is a major muscle attachment site. As such, the three areas of interest for the subperiosteal equation are anterior, medial, and lateral. As with the Kerley-Ubelaker method, the field size was placed onto a calibrated image captured by Sigma Scan Pro 5.0 through the Leica DM 2500 light microscope. Three areas (circular field size) of each aspect (anterior, medial, and lateral) were then analyzed. The number of present Haversian canals were counted within the given field size. If a canal was only half present, it was counted as one half in the tally. If the Haversian canal was irregularly shaped, practically destroyed, or the diameter was greater than 200 microns (γm), it was eliminated from the counts total. The three areas of each aspect were then averaged, multiplied by the CF, and entered into the periosteal surface equation (HPA).

Table 2. Regression equations for Hauser et al. (1980) age at death estimation
(Y = Age in years; X = Haversian canal count)

Variable	Formula
Hauser-P Age (HPA)	$Y = 4.86X - 22.22$
Hauser-E Age (HEA)	$Y = 7.96X - 45.49$
Hauser-Average (HAA)	$Y = \frac{(4.86X - 22.22) + (7.96X - 45.49)}{2}$

Endosteal Surface

The posterior, medial, and lateral aspects of the endosteal surface of the femoral midshaft were analyzed. Three areas (circular field size) of each aspect were then examined through the quantification of present Haversian canals. This was followed by

the average of counts of each aspect analyzed. Additionally, the same constraints for the periosteal equation remained (i.e. one half of a canal present was counted as one half and irregularly shaped Haversian canals, those practically destroyed, and those with a diameter greater than 200 microns (γm) were eliminated). The average of the aspects themselves (i.e. posterior, medial, lateral) were then determined, the CF applied, and entered into the endosteal equation (HEA).

Intra-observer error

For the purposes of this research, intra-observer error was also tested. Lynnerup suggested that the tally of osteons was likely to be consistently identified (1998). Past research suggests that high rates of intra-observer error with osteon fragments and Haversian canals are more likely than low rates. As noted earlier in this thesis, however, the error rates for the identification of Haversian canals as the sole feature of interest were not analyzed (Lynnerup et al., 1998). As such, the estimate of likely intra-observer error cannot be stated at this point for the identification of Haversian canals. As each slide was analyzed twice, it was decided that, because of the additional experience provided by the first estimation, the second set of estimations would be the one involved in the statistical analyses (except for analysis of intra-observer error).

Statistical Analyses

A database was created on SPSS 14.0 containing burial number, sex, chronological age at death, corrected Kerley-Ubelaker osteon age, corrected Kerley-Ubelaker osteon fragment age, Kerley-Ubelaker non-Haversian canal age, Kerley-

Ubelaker percentage of lamellar bone age, Kerley-Ubelaker osteon/osteon fragment average age (KUA), Hauser et al. periosteal surface age (HPA), Hauser et al. endosteal surface age (HEA), and Hauser et al. endosteal/periosteal surface average age (HAA). In order to better understand the relationship between the chronological age of individuals and the estimates created by each histological method (KUA, HPA, and HEA), Pearson's r correlations were run through SPSS. Additionally, in order to determine intra-observer error, the Pearson's r correlations were run comparing the first attempt of each technique with the second. Following the accepted rate of error reported by Weinberg et al. (2005), intraobserver error measurements greater than $p = .05$ (5%) are considered imprecise for the purposes of this analysis.

The correlation coefficient "expresses the ratio of the covariance in x and y to the product of the variation in x and the variation in y" (Bachman and Paternoster, 2004; p. 462). In other words, it indicates the magnitude and direction (positive or negative) between the variables compared. The more similar the actual age at death is with each estimate, the more similar the variation of x and of y. Bachman and Paternoster (2004; p. 462) report that "a correlation has a lower limit of -1.0 and an upper limit of +1.0." If the real age and estimated age are equal, the Pearson's r correlation will be 1.

Paired sample t-tests were also run through SPSS 14.0. The intent was to determine if the means of the real age from the Ubelaker slide sample significantly differed with each method's (KUA, HPA, HEA, and HAA) estimated age. Paired t-tests were also run to determine if a statistically significant difference is present among KUA, HPA, HEA, and HAA as well as to examine intra-observer reliability. Paired sample t-tests can only be used with matched pairs (Bachman and Paternoster, 1997). Matched

groups or pairs t-tests are based on dependent samples in which the "cases in one sample are deliberately selected so that they are comparable to the cases in another sample" (Bachman and Paternoster, 1997; p.667). A paired t-test was used as the variables compared were the estimated and real ages of the individuals from the Ubelaker sample. As such, they were both dependent on the growth and development and/or the rates of degeneration for each of the individuals analyzed in this research study. The t-test of the matched group then tested the difference between the means for each pair (x = mean; x_2 - x_1).

The pairs analyzed were contrasted and examined in order to understand the statistical significance between the real ages with each of the methods for age estimation (Kerley-Ubelaker, Hauser-P, Hauser-E, and Hauser-Avg). Upon the completion of the initial age estimations, it was noted by the author that the youth of some individuals (9-21) reflected, in some methods, highly inaccurate age estimations. As such, t-tests were performed on the complete sample population with 29 degrees of freedom (N=30) and a subset of the sample with 24 degrees of freedom (n = 25). This subset included individuals older than 21 and was analyzed in order to more accurately estimate the effect of extreme values of on the aforementioned methods.

Chapter 7: Results

Sample Results of Question 1

Research Question 1 asks whether the level of correlation between the reported actual ages of the individuals at death included in the Ubelaker slide sample analyzed by the various age estimation methods is equal to 1.0. The Pearson's r correlation was run through SPSS 14.0 between real ages at death of the Ubelaker slide sample (RA) and the ages estimated by the Kerley-Ubelaker methods, the equations for Hauser-P (HPA) and Hauser-E (HEA) as well as the average estimation created by averaging Hauser-P and Hauser-E (HAA). When analyzing Pearson's r, a value of -1.0 is equal to a perfect negative relationship, a 0.0 value indicates no relationship between the two continuous variables, and a correlation of 1.0 r value is a perfect positive relationship (Bachman and Paternoster, 2004). The correlation itself provides an assessment of the strength of the linear relationship between two variables.

The correlation between RA and KUA is .652, which is positive (Appendix B, Table 3). This means that the higher the RA of the individual, the higher the reported KUA. The correlation is significant at the .05 level. The correlation between RA and age estimation of Hauser-P (HPA) is equal to .690 ($p < .05$), a slightly stronger positive correlation with real age than that of Kerley-Ubelaker (Appendix B, Table 4). The results of the RA and HEA correlation is slightly lower than that of both KUA and HPA with r equal to .585 (Appendix B, Table 5), but is still significant at the .05 level. The correlation between RA and the average of HPA and HEA (HAA) is .679, also significant at the .05 level (Appendix B, Table 6). While the significance level for correlations were all significant at the $p=.05$ level, all methods are also strong enough to have significant

correlations related to p=.01. These results indicate that a strong and positive relationship exists between the actual age at death and each of the estimates.

Sample Results of Question 2

Statistical significance is the topic of Research Question 2. Paired sample t-tests were conducted to indicate whether a statistically significant difference could be found in the overall age estimations for each of the equations and methods utilized: Kerley-Ubelaker (KUA), Hauser-P (HPA), Hauser-E (HEA), and the total average age determined by both Hauser equations (HAA). For this research question, the significance level is the probability of rejecting the null hypothesis that no mean difference exists between the real ages and the age estimations from each method. All t-tests performed were two-tailed with p-values of .05. Each p-value measures the strength of evidence *against* the null hypothesis, representing the probability that a value will reach or exceed the given data point under the null hypothesis (Johnson and Bhattacharyya, 2001). The smaller the p-value identified, the stronger the evidence for and the higher the likelihood that the results have a statistically significant difference.

Also of interest is the overall directionality of each method. The null hypothesis states that there would be no statistical difference between the mean numbers of years estimated from real (RA) by the Kerley-Ubelaker method (KUA). The alternative hypothesis suggests a difference in the mean number of years for each variable, indicating a significant over- or under-aging. If a statistically significant difference is found, the method would not be an accurate age estimator of the Ubelaker slide sample and would indicate that the result is unlikely to have occurred by chance (Johnson and

Bhattacharyya, 2001). However, a significant difference does not necessarily imply that the difference between the means is large (Bachman and Paternoster, 2004). If no significant difference is found, we would conclude that the Kerley-Ubelaker method is an accurate estimation of age when examining the Dominican Republic Ubelaker slide sample.

When testing the difference between the Kerley-Ubelaker method and real age, the paired sample t-test indicated a mean age of 42.10 years for RA and 49.13 years for KUA (Appendix B, Table 7). This is a 7.03 year difference between the two means, indicating that the estimate derived from Kerley-Ubelaker is (on average) consistently larger than the real age. Of the ages estimated by KUA, 23 percent of the estimated ages were within 5 years of the actual age at death, 46 percent were within 10 years, and 95 percent were within 30 years (Appendix B, Table 23). The average from RA is 13.69 years (Appendix B, Table 8). This means that when looking at the difference in absolute values between the actual age at death and the estimated age for the sample of 30 individuals, the difference is 13.69 years. As is reflected by Table 8 in Appendix B, the value of t at 29 degrees of freedom was -2.46. The t-value was then entered into the t distribution in order to determine the level of significance. Since the two-tailed significance level was .02, which is less than .05, there is a statistical difference between the means, and the null hypothesis was rejected at the p-value of .05. Therefore, Kerley-Ubelaker is not an accurate method of estimating age for the Ubelaker slide sample.

In order to determine whether the age of the sample affected the overall age estimation and statistical significance of the Ubelaker slide sample, a paired sample t-test was conducted including those only older than 21 years of age at death. The null and alternative

hypotheses were otherwise identical to those mentioned previously. In Appendix B, Table 9, the mean ages were adjusted to 47.04 years for RA and 52.09 years for KUA. The mean difference is noticeably smaller than in the first analysis, when individuals younger than 21 years at death were included. The difference between RA and KUA decreased from a 7.03 to 5.05 years, and was not statistically significant (p=.12) (Appendix B, Table 10). The percentage distance from the chronological age at death remained similar (Appendix B, Table 24) with 24 percent, 44 percent, and 95 percent within 5, 10, and 30 years from real age, respectively. As such, by excluding those 21 years of age at death or younger, the Kerley-Ubelaker method as an age estimator is accurate.

Utilizing the same null and alternative hypotheses reflected above, the author found that the mean age using HPA for n=30 was 46.73 and was also higher than the RA mean of 42.10 (4.63 years) (Appendix B, Table 11). It was also revealed that 26 percent of the age estimations by HPA are within 5 years of the reported ages at death of the Ubelaker sample as is displayed in Table 23, Appendix B. Further, 43 percent were within 10 years and 95 percent were within 30 years of the actual age. The average error from RA for HPA was 13.81 years. The null hypothesis was not rejected because the mean difference in years was not significant as p=.16 (Appendix B, Table 12). Therefore, when analyzing the Ubelaker sample of slides from the Dominican Republic, the Hauser-P equation is an accurate method for age estimation.

When examined with a smaller subset of individuals, and once again with the same null and alternative hypotheses stated above, the HPA mean age was approximately 4.82 years older than mean age of RA (Appendix B, Table 13). The percentage of difference from the actual age at death by the HPA estimate was similar to the previous

results: 28 percent within 5 years, 25 percent within 10 years, and 95 percent within 30 years of the reported age at death of the Ubelaker slide sample (Appendix B, Table 24). This difference is very similar to that of RA and HPA with the complete, 30 individual sample. In Table 14 of Appendix B, a significance level of .24 is reflected. It is again greater than the .05, and, therefore, an accurate method for age estimation of the Ubelaker sample.

The mean difference in years for the endosteal surface of bone was 52.03, 9.92 years above that of RA, 42.10 (Appendix B, Table 15). When examining the accuracy of each estimate that 13 percent of the slide samples were within 5 years of the reported age at death, 30 percent were within 10 years, and 95 percent were within fifty years. Appendix B, Table 23, reports this data. Additionally, the average error was found to be high at 19.60 years (Appendix B, Table 16). As p=.02, a significant difference was found between RA and HEA. This indicates that HEA is not an accurate method for estimating age for the complete sample of the Dominican Republic Ubelaker slides.

When excluding individuals aged 21 and younger and reflecting the previously stated hypothesis, the mean of HEA was 57.35, still approximately 10.31 years greater than that of RA, 47.04 (Appendix B, Table 17). Unique to this method, with the smaller sample excluding the youngest five individuals from the Dominican Republic slide sample, the accuracy decreased. Only four percent (i.e. n=1) of the sample was estimated to be within 5 years of its actual age, and 24 percent were within 10 years (Appendix B, Table 24). On the other hand, 95 percent of the age estimations remained within 50 years of the reported ages at death. A significant difference was found (p=.04) (Appendix B, Table 18). Consequently, while age does not seem to be a factor in the overall estimation

of HEA, it is still not an accurate method in determining age at death for the Ubelaker slide sample.

A paired sample t-test was also performed for the average age estimations of both Hauser equations (HAA). Again, the null hypothesis states that there will be no significant difference in the mean number of years estimated from real for the Hauser-Avg. The difference between the means of HAA and RA was 7.28 (Appendix B, Table 19). Distances from the actual ages at death of the Dominican Republic slide sample are reported in Appendix B, Table 23, were 23 percent within 5 years, 43 percent within 10 years, and 95 percent within 40 years of the actual age at death. The t-value was -2.23 with 29 degrees of freedom. The average error rate was found to be 14.06 years (Appendix B, Tables 20). A statistically significant difference was noted (p=.04) and the null hypothesis was rejected. The average Hauser method was not found to be an accurate estimator of age at death for the complete Dominican Republic Ubelaker slide sample.

When examining HAA in relation to individuals older than 21 years at death, the Hauser mean was 54.34, 7.30 years greater than 47.04 (Appendix B, Table 21). This is slightly larger than the mean difference when compared with that of the complete sample. The percentages of the ages estimated within 5, 10, and 40 years from the reported ages given in the Ubelaker slide sample, were 20, 40, and 95 percent, respectively (Appendix B, Table 24). The t-value was reported, as is shown in Appendix B, Table 22, to be -2.00 and the level of significance supported the null hypothesis at .06. As such, the Hauser-Avg method, when taking into account individuals older than 21 years of age at death in the Ubelaker sample, is an accurate age estimator.

The second part of Research Question 2 was focused on the overall directionality of each method. It was found that all of the methods used to estimate age (KUA, HPA, HEA, HAA) reflected negative mean differences, indicating a consistent overestimation of the age of the sample individuals as a whole (Appendix B, Table 25). As such, there was a clear, positive directionality to all of the methods. The differences ranged from 4.63 (HPA) to 9.93 (HEA) years difference.

When examining the mean differences for the sample subset of 25 individuals older than 21 years, the overestimation persists. The age estimation methods of the highest and lowest mean differences from real remain the same as with the complete sample of 30. The differences range from 4.28 (HPA) to 10.31 (HEA) years (Appendix B, Table 25, Table 26). KUA reflects a smaller mean difference from 7.03 down to 5.05, a difference of almost two years. The Kerley-Ubelaker method (1965, 1978) seemed to clearly indicate a vulnerability to individuals 21 years of age or younger at death. The equations developed by Hauser et al. (1980) were not affected as extremely, with HEA gaining a mean difference, in years of .39. HPA, in difference, grows closer to the RA (as with KUA) by .35 years. As HEA reflected a negative movement from the complete sample analysis to the smaller age-affected subset and HPA displayed a positive movement of roughly the same distance, the difference between the two samples reflected an adjustment of only .02 years for HAA. For the purposes of this analysis, all Hauser equations are less affected by youth than those of Kerley-Ubelaker.

Sample Results of Question 3

The first part of Research Question 3 examines the correlations between the methods of age estimation themselves. The table containing the results of each of these

analyses can by found in Appendix B, Table 27. When comparing KUA and HPA, the r value is high at .910. This indicates a very strong positive correlation between the estimations of age by KUA to HPA. The correlation between KUA and HPA is almost 1.0, indicating a nearly perfect positive correlation with little difference indicated. The r value for KUA and HEA, however, is much lower, although still strong at .688. The Pearson's r correlation between KUA and the total Hauser equations of HAA is, at .847, also strongly positive. When examining the correlations of the methods with HPA, the r value with HEA was found to have a strong positive correlation at .740 and a very strong positive correlation with HAA at .922. Pearson's r value is the highest and strongest when comparing HEA and HAA at .943. As HAA is the average of HPA and HAA, this correlation and lack of differentiation is expected. All intercorrelations were found to be significant at $p = .05$. These correlations are also strong enough to be significant at $p = .01$.

In order to measure the level of significant differences among KUA, HPA, HEA, and HAA for the Ubelaker's Dominican Republic sample, paired t-tests were run through SPSS 14.0. The null hypothesis for each held that no statistically significant difference would be found between the mean values of each method. The alternative hypothesis would reflect a statistically significant difference between the values of the methods. These hypotheses were found to be valid for all analyses under Research Question 3. If the level of significance was found to be lower than the p-value of .05, the null hypothesis would be rejected and would note a statistically significant difference between the variables (methods).

The differences between the age estimated by Kerley-Ubelaker (KUA) and all possibilities of those estimated by Hauser (HPA, HEA, HAA), were of primary interest. Are

the two methods significantly different from one another? KUA and HPA reflected a mean positive difference of 2.40 years, generally estimating age as slightly higher than Kerley-Ubelaker (Appendix B, Table 28). The t-value was 1.3 with 29 degrees of freedom. The significance level of this two-tailed test was .22, larger than the pre-stated p-value of .05, so the null hypothesis was not rejected (Appendix B, Table 29). As reflected in Appendix B on Table 30, the t-test was again run comparing KUA with HEA, indicating a mean negative difference of -2.90 years. Resultantly, the endosteal Hauser equation will be likely, with the Ubelaker slide sample, to underestimate those of Kerley-Ubelaker by almost three years. The significance level was .44, well over the p-value and, once again, the null hypothesis fails to be rejected (Appendix B, Table 31).

The mean difference is noticeably affected when comparing KUA with HAA (the average of HPA and HEA). As the KUA - HPA mean difference was slightly positive, the KUA - HEA mean difference was slightly negative, and the distance for the differences were very similar, the mean difference between KUA and HAA was only -.25 years (Appendix B, Table 32). The t-value was -.11 for the 29 degrees of freedom and, as displayed on Table 33 in Appendix B, the significance level was high at .92. No statistically significant difference was found between the age estimations of Kerley-Ubelaker and Hauser for the Ubelaker sample. Also reflected in the significance levels is the fact that the average of the two Hauser equations is most similar to those of Kerley-Ubelaker.

Paired sample t-tests were then run to identify statistically significant differences within the Hauser method. HPA and HEA had a negative mean difference of -5.30 years (Appendix B, Table 34). The t-value was -1.53 at 29 degrees of freedom (Appendix B, Table 35). The p-value remained .05 and was again lower than the significance level of

.14. The null hypothesis was not rejected and no significant difference between the endosteal and periosteal age estimations found. As HPA and HEA are averaged together, the significance levels for each when compared to HAA are equal at .14, again greater that .05 (Appendix B, Tables 37 and 39). The t-values were inversely equal at -1.53 (HPA/HAA) and 1.53 (HEA/HAA) (Appendix B, Tables 36 and 38). All Hauser methods have the same significance level of .14. As expected, there were no statistically significant differences found between Hauser-P and Hauser-E.

Correlations examine the linear relationships between two variables. On the other hand, statistical significance endeavors to address the specific idea proposed by each hypothesis. The significance level is the probability of rejecting the null hypothesis when it is true. The null hypothesis in this scenario holds that no statistically significant difference will be found among KUA, HPA, HEA, and HAA. While correlations can give one an idea of the overall relationship between two variables, it cannot state the level of significance between them. This is reflected by the analyses for Research Question 3. For example, the correlation between KUA and HPA was .910, and the t-test reported a significance level was .217. Alternatively, KUA and HEA was .847 while the paired t-test had a significance level of .917. The differences between correlation value and significance level indicate that while the 1 to 1 correlation between KUA and HPA may be higher than that of KUA and HEA, so, too, is the likelihood of incorrectly rejecting the null hypothesis. This author believes that these differences are due to the small sample size included in the study.

Sample Results Question 4

Pearson's r correlations were run to determine the intra-observer error reliability of the author. Each age estimation (KUA, HPA, and HEA) was performed twice. The second estimation was utilized for the previous analyses. Each estimate will be termed 1 or 2 respectively in accordance with the first and second analyses [Kerley-Ubelaker Age 1 (KUA1) and Kerley-Ubelaker Age 2 (KUA2), Hauser-P Age 1 (HPA1) and Hauser-P Age 2 (HPA2), Hauser-E Age 1 (HEA1) and Hauser-E Age 2 (HEA2), Hauser-Avg Age 1 (HAA1) and Hauser-Avg Age 2 (HAA2)]. The correlations were determined in the same manner as Research Question 1. The Pearson's r value between Kerley-Ubelaker Age 1 (KUA1) and Kerley-Ubelaker Age 2 (KUA2) was determined to be .907 (Appendix B, Table 40). This is an especially strong positive correspondence. The correlations between HPA1 and HPA2 was also very strong with an r value of .926 (Appendix B, Table 41). The r value for HEA1 and HEA2 is slightly lower though still a very strong positive correlation of .864 (Appendix B, Table 42). HAA reflects an additional very high correlation between HAA1 and HAA2 with an r value of .913 (Appendix B, Table 43). All correlations are significant at the .01 level, well within the acceptable level of p =.05 discussed by Weinberg et al. (2005).

Chapter 8: Discussion

All methods of age estimation examined in this study reflected significantly positive correlations with the actual age of the individuals. The correlations show the relationship of variables to one another but are not useful for indicating the overall accuracy of each method. The correlations are all positive. This indicates that as the real age (RA) of an individual increases, then, generally, so do each of the age estimation methods (KUA, HPA, HEA, and HAA). The rank correlations of the methods from lowest to highest are HEA, KUA, HAA, and HPA. HPA has the highest r value for the Pearson's r correlation at .690. The lowest value results from the HEA equation at .585. HAA is close to the r value of HPA at .679.

The results of the paired sample t-test varied. When examining each method for the complete sample of 30 individuals, the Kerley-Ubelaker method, the Hauser-E, and the Hauser-Avg equations were not found to be accurate with statistically significant differences present in the mean values for each. The Hauser method, when utilizing the equation of the periosteal surface of the bone, exhibited no statistically significant difference in the mean number of years from real. When compared to the RA, directionality was present as well as both strong and in a positive direction for all of the equations involved in the statistical analyses (KUA, HPA, HEA, and HAA). This means that for the sample analyzed, it is more likely than not that the age estimation for each of the methods will be older than that of the actual individual.

By eliminating the youngest individuals from the age estimations (5 individuals aged 9-21 years at death), results generally tended to be more accurate. There is no statistically significant difference for the Kerley-Ubelaker method when utilizing the

Ubelaker slide sample, and is thus an accurate method for estimating age. The Hauser-P and Hauser-Avg equations were likewise accurate. However, the Hauser equation based on the endosteal surface of bone had a statistically significant difference between the mean ages of real and HEA. This resulted in an inaccurate age estimation for the given Dominican Republic sample. This makes sense as the endosteal surface of bone is mainly a site of bone resorption after the ages of 30-35 years (Eriksen, 1994; Martin, 1998). As a result, it would be expected that Hauser-E would be most accurate when the individuals analyzed are 35 years of age or younger. This is in direct contrast to the results found with the Kerley-Ubelaker method, which reflected a vulnerability to bias with a younger sample set.

Discussed in the previous section, the mean difference in years is the average for the *total* of the respective age estimations minus the average for the *total* of the actual ages at death. The average error in years is the absolute value of the distance for *each* estimated age from the real age at death of *each* individual. For example, an age estimation of 42 for a 50 year old individual has an absolute distance of 8 years. Thus, while the mean difference is focused on the total group average, the average error is concentrated on the individual.

In 1981, Ubelaker estimated the ages of individuals to have an average error of 10.43 years using Kerley-Ubelaker for the complete sample analyzed (N=114) (1981). This researcher has found the average error of the subset sample (n=30) for KUA to be 13.69 years (Appendix A, Table 8). Reasons for this difference could very well lie in the decreased sample size. It is also possible that the use of osteons and osteons fragments as the variables to quantify affected the estimations. As Ubelaker did not mention which

variables (osteons, osteon fragments, non-Haversian canals, and percentage of present lamellar bone) were used in the original Kerley-Ubelaker analysis, it is not known whether this is a valid factor. The Hauser-P is similar to that of Kerley-Ubelaker with an average error of 13.81 years (Appendix A, Table 12). Hauser-Avg had a similar average error at 14.06 years as is reflected in Table 16 of Appendix A. The highest average error came from Hauser-E at 19.60 years (Appendix A, Table 20).

Statistically, the Hauser-P method was found overall to be most accurate with the Ubelaker slide sample, as it is not different in a manner that is statistically significant from the mean difference of the actual age of individuals. This holds true for both the full sample (n=30) as well as the sample subset (n=25) of individuals older than 21 years. Both the Kerley-Ubelaker method and the Hauser-Avg equations were not found to be accurate with the initial sample of 30 but became more accurate when younger individuals were taken out of the pool for analyses. There is a key difference to note, however, as the p-value of KUA moves from .02 to .12. This is a large jump in significance and indicates that those included in the sample that were 21 years of age or younger had a significant effect on the accuracy of age estimations. Conversely, HAA's p-value is much smaller, moving from .03 to .06. This indicates that while youth may have affected the overall estimation of age, this effect was relatively small. The Hauser-E equation was found not to be accurate at both sample levels (N=30 and n=25). What is interesting about the statistical results for this equation is that, unlike all of the others, the full sample reflects a smaller mean difference from the subset of ages for individuals. The overall difference between the significance levels for the two sample sizes, however, was the smallest of all methods analyzed at .02 (.02 for N=30 and .04 for n=25).

The comparison among methods was performed in order to gain insight into the similarities between the Kerley-Ubelaker (1975; 1978) and Hauser (1980) methods themselves. Also of interest was the level of significant difference between the two Hauser equations. Pearson's r correlation was initially run, comparing KUA, HPA, HEA, and HAA among one another, which exhibited interesting results. Although the initial correlations for KUA and HPA with RA were .65 and .69 respectively, when correlated with each other, the r value was incredibly strong at .91. The lowest correlations among the equations, while still strong, were both related to that of Hauser-E (HEA/KUA, .69; HEA/HPA, .74). When the paired sample t-test was utilized to examine the similarity amongst Kerley-Ubelaker, Hauser-P, Hauser-E, and Hauser-Avg, the value of significance in mean difference was low, keeping with hypotheses stating no significant difference in the mean values of each age estimation existed. An interesting significance level occurred between the two methods themselves (i.e. the endosteal and periosteal equations of Hauser were averaged) as the significance level was .92.

The correlation rates were also utilized in order to determine the intra-observer error rates. A p-value of .05 was chosen as a statistical limit measuring intra-observer error (Weinberg et al., 2005). The correlation between the first and second analysis is strong, with each of the methods at a value of .01, more accurate than that limit given. The highest correlation is with the HPA method at .93 while the lowest is with HEA at .86. Since both HPA and HEA consist of counts of present Haversion canals, the consistency of their identification on the Ubelaker slides appears to be variable. As HPA is the most consistent correlation method, it is indicative that the identification of

Haversion canals for the Hauser method on the subperiosteal surface of bone is straightforward and simple.

What are some of the possible reasons responsible for differentiation among the results, and what factors may have affected the estimations of age for each of the methods? As was stated and addressed in the results section of the statistical analyses and at the beginning of this discussion, subadult aging seems to have affected the overall accuracy for the Kerley-Ubelaker method. Indeed, past research has suggested that a subadult skeleton has been thought to be too complex for analysis due to the stacking of bone modeling and remodeling for cellular based structures (Stout, 1992).

Another possible affecter of age estimations is sample size. Although the size of the sample utilized for this study is larger that some, such as Ahlqvist and Damsten (1969) with a sample of 20, Stout and Gehlert (1980) with 13, and Paine and Brenton (2006) with 26 individuals, these smaller samples were present mostly for review of methods rather than a complete analysis. A greater sample size would tend to "normalize" the population and reduce the effects of bias and outliers on the overall estimations themselves due to a multitude of variables, some of which are listed below.

The level and rate of mechanical loads of the individuals analyzed in the Ubelaker sample from the Dominican Republic is thought to be high as the population was generally labeled as laborers (1981). As such, the rate of physical activity for these individuals would be expected to be high. The rates of activity for the contemporary American sample upon which the Kerley methods is based and the contemporary French sample upon which Hauser et al. is based, whether sedentary or active, possibly played a role in the age estimation for each of the methods. Chan et al. (2007) cautions against

use of the posterior area of the femur in determining age due to muscle connections at the linea aspera. The periosteal surface of the Hauser method (1980) does not include the posterior aspect of the femoral midshaft specifically in order to avoid a skewed age estimation result. As Ubelaker (1974) mentioned, a discrepancy is possible due to unequal activity levels among populations, specifically that of the Dominican Republic sample and those from which the Kerley-Ubelaker (1965; 1978) and Hauser et al. (1980) methods are based.

Finally, variations among the ancestral populations themselves could also be a key to the differences in age estimation for the Kerley-Ubelaker (1965; 1978) and Hauser et al. methods (1980). While Kerley did not mention the specific sample in the original creation of the equations, it was reported that there was an overall success in applying his method to skeletal remains from varying populations. These included testing archaeological samples from Native American skeletons from Kentucky, Florida, and Virginia, as well as the Aleutian and Philippine Islands with dated ages at death from 500 to 5,000 years before the time of analysis (1965). Holding with the results found in this study, Ubelaker contested this success, reporting that the method produced high standard errors with an average of 11 years from the femora of individuals from the Dominican Republic who were classified as "Black or mulatto" (Ubelaker, 1978). The sample for the Hauser et al. method was also based on contemporary cadavers, specifically French (no further ancestral details given) (1980).

Chapter 9: Conclusion

The goal of this study was to test the overall Hauser et al. method (1980) as a usable estimator of age at death. A sample of 30 individuals was collected to better understand both the relationship between the Hauser (1980) and the Kerley-Ubelaker (1965; 1978) methods of age estimation as well as to examine the accuracy and similarities between the subequations for the endosteal and periosteal surfaces. This study examined how Hauser et al. compares with a known and generally accepted age estimator, Kerley-Ubelaker (Kerley, 1965; Kerley-Ubelaker 1978; Ubelaker, 1981; Ahlqvist and Damsten, 1969; Robling and Stout, 2000). The Hauser method itself is a compilation of two separate equations based on the endosteal and subperiosteal surfaces of bone that the original authors suggest are successful for independent use. Additionally, the accuracy of each of the methods or equations [Kerley-Ubelaker (KUA), Hauser-P (HPA), Hauser-E (HEA), and the average of both Hauser equations (HAA)] at estimating age, was also examined. Pearson's r correlations and paired sample t-tests were run through SPSS 14.0, comparing each method or equation (KUA, HPA, HEA, and HAA). This research was performed in order to more closely examine how Kerley-Ubelaker (1978) and Hauser et al. (1980) compare as methods, regardless of the accuracy of the age estimation, with the Ubelaker sample.

When the above methods were examined for accuracy, preliminary results suggested skewed or inaccurate age estimations of younger individuals (RA$\leq$ 21 years at death). As such, a second set of paired sample t-tests were run on a smaller sample that excluded individuals from the Ubelaker slide sample who were equal to or younger than 21 years at death. This reduced the slide sample to n=25.

It was expected that the Kerley-Ubelaker method would both overestimate and underestimate the age of the slide sample utilized from Dominican Republic (Ubelaker, 1981). This was found to be true; however, KUA as a whole tended to overestimate the ages of individuals. It was unknown whether the Kerley-Ubelaker method would have statistically significant mean differences. The expected outcomes were also unknown for each of the t-test analyses when examining the accuracy of each of the Hauser (1980) estimations (HPA, HEA, and HAA). When observing the intercorrelation of each method or equation, no expectation was present on the similarities of each method. Intra-observer error had no preconceived expectations as this author has no past history of recording intra-observer error.

Hauser et al. (1980) introduced a method that can estimate age at death based on counting the Haversian canals in two separate locations: subperiosteal and endosteal. When estimating the age of individuals in the Ubelaker slide sample, the subperiosteal equation reflected both the highest rate of accuracy of all methods analyzed and the highest correlations (of KUA, HPA, HEA, and HAA) to real age at death. The subperiosteal equation for Hauser was determined to be accurate for the Dominican Republic sample; however, the intra-observer error rates were estimated to be less accurate. The higher presence of both Haversion canals and osteon fragments could explain this and is consistent with results found by Lynnerup et al. (1998).

Conversely, the endosteal surface of the Hauser method was the least accurate and displayed the lowest r value when analyzed against the actual age at death. These results are significantly different from those reported by Hauser et al. (1980) who stated that the equation of endosteal surface was more accurate than that of the subperiosteal. A strong

advantage of the Hauser et al. method could lie in its endosteal equation. When the exterior of bone is damaged and unable to be analyzed, there are few methods of age estimation that utilize the endosteal envelope of bone. Forensic anthropology and bioarchaeology could both benefit from continued research in age estimation methods utilizing the endosteal surface of bone. The sample used in this study represents underprivileged individuals identified in records as "black" or "mullatto" (Ubelaker, 1981). Although a statistically significant difference was found between the actual age of the individuals at death and the endosteal Hauser equation, this author hypothesizes that the differences in physical activity and/or diet is largely responsible for the significant mean differences.

In this thesis, Hauser et al. has been found to be a valid age estimator and more accurate than that of Kerley-Ubelaker when applying the periosteal surface equation (HPA) and the overall average of the Hauser equations (HAA) on the Dominican Republic slide sample. Unfortunately, the endosteal equation of Hauser (HEA) is not an accurate method of age estimation with the sample provided by Dr. Ubelaker. Differences in these variables are factors that affect the rates of bone resorption and formation along the endosteal surface of bone. As such, the endosteal surface equation of Hauser et al. should be examined using a similar population as was initially utilized in its development (1980).

I would not necessarily discount the use of the endosteal equation, however, as the correlations between the actual age of individuals and age estimation provided by the endosteal equations have a strong positive relationship. Instead, I would simply caution its use when other methods of age estimation are available. The factors considered most

responsible for differences, according to this author, are small sample size, non-similarity between sample populations for the Dominican Republic and that upon which the Hauser method is based, as well as the age of the individuals at death. Future research should examine the endosteal surface of bone utilizing a larger, European-based contemporary sample of individuals 35 years of age and younger.

When comparing the Kerley-Ubelaker method to that of Hauser-Avg age, the correlations were very high, and the mean difference between the methods was less than a quarter of a year. The methods' results were nearly identical and indicate a great similarity between the two. As a next step in comparing the two methods, this author suggests the Hauser method be directly compared to that of Kerley-Ubelaker using a contemporary population that is either American (original Kerley sample) or French (the Hauser sample) (Kerley, 1965; Hauser et al., 1980).

Future research should also specifically examine histological differences between sexes. While this study did not test for differences between the sexes citing past research (Kerley, 1965; Stout and Paine, 1992; and Stout et al. 1994), some studies have reported differences in accuracy rates of estimating age between the sexes (Thompson 1981; Ericksen 1991). Though not included in this thesis, the original Hauser et al. (1980) article also included a method of age estimation based on the tibia. Although it was reported to have a lower level of accuracy than the femoral Hauser method, the tibia could offer a useful alternative in instances when the tibia is the only bone present for age estimation.

Appendix A

Figure 1 – Shaft of Long Bone. Displays Examples of Haversian Systems and Volkman's Canals (Above). The Lower Image on the Right Exhibits a Magnified View of an Osteon. Adapted from Junqueira and Carneiro (2005).

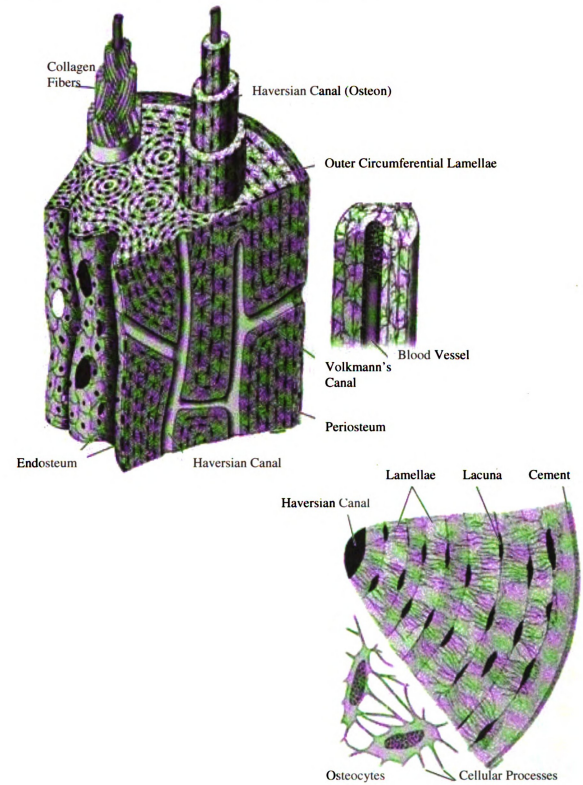


Figure 2 – The Longitudinal Aspect of a Basic Multicellular Unit (BMU) in Action, From Right to Left, Representing the Formation of an Osteon. Adapted from Robling and Stout, 2000).

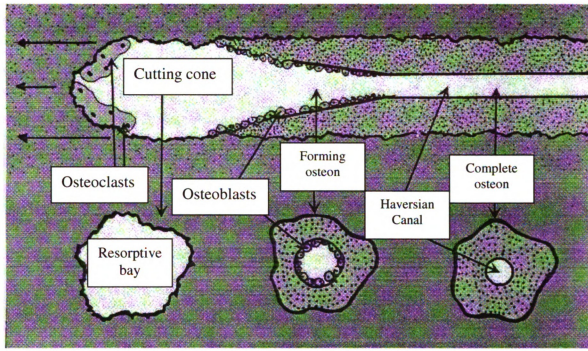


Figure 3 – From Left to Right, Dell Dimensions 4550 Computer, Sony Camera Adapter CMA-D2 on Top of a Sony Trinitron, and a Leica MZ 12 Stereomicroscope Attached to a Computer. Sigma Scan Pro 5.0 Program is on Screen of Computer. Michigan State University, Forensic Anthropology Laboratory.

Figure 4 – Example of Anterior, Medial, Lateral, and Posterior Areas of Bone Used in Analysis of Femoral Midshaft in Kerley-Ubelaker Method (1978).

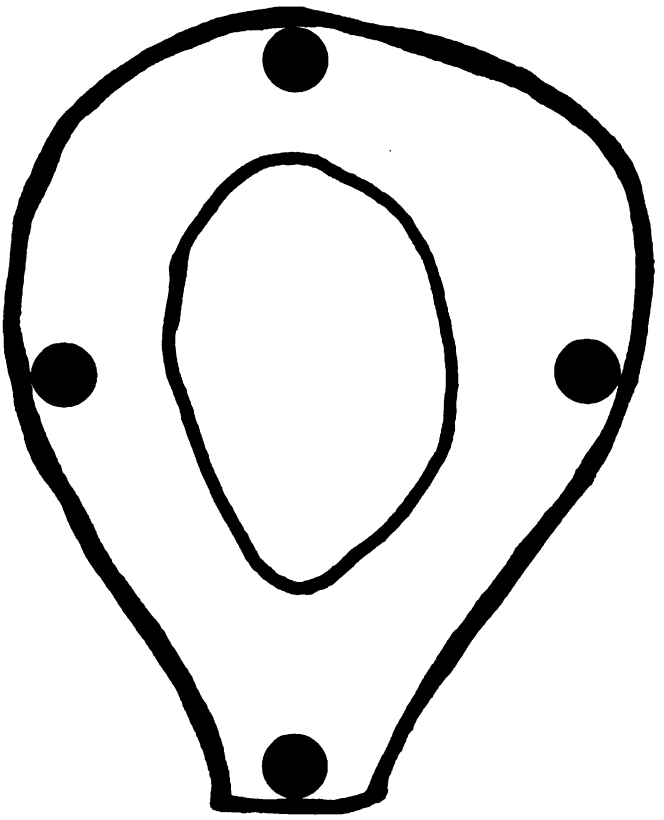


Figure 5 – Example of Fragmentary Osteon (Outlined in Red) on a Digital Image at 200x in Association with an Intact Osteon (Indicated by Arrow). Adapted from Tersigni (2005).

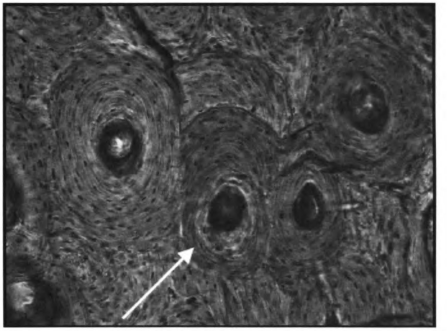


Figure 6 – Example of Kerley-Ubelaker (1978) Analysis. Circles are Identified Haversian Canals (10 present) and Lines Indicate Osteon Fragments (5 present). Image Not Used in Analysis.

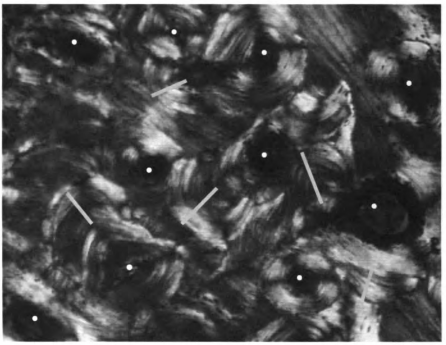


Figure 7 – Periosteal and Endosteal Surfaces of Human Bone. Macroscopic view (Left) of Femoral Midshaft Showing the Periosteal and Endosteal Bone Surfaces. Microscopic View (Right) of Human Rib (100 X) Showing the Periosteal and Endosteal Surfaces (right). Adapted from Tersigni (2005).

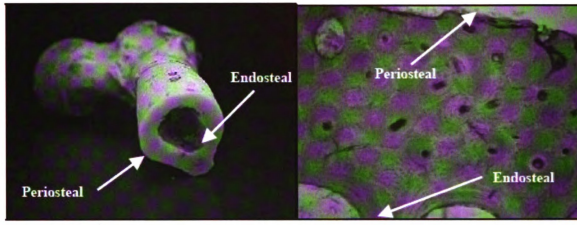


Figure 8 – Example of Areas of Bone Used in Analysis of Femoral Midshaft in Hauser et al. Method (1980). P= Periosteal Equations (Three Averaged Areas Each of Anterior, Medial and Lateral) and E= Endosteal Equations (Three Averaged Areas Each of Anterior, Medial and Lateral).

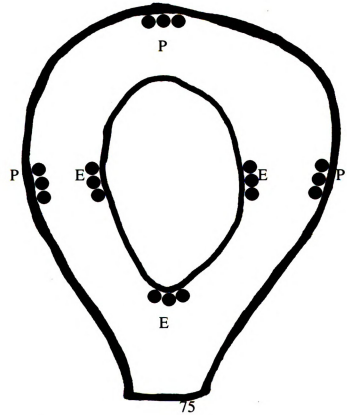


Figure 9 – Microscopic (400x) View of Haversian Canal (Arrow) of a Human Tibia. Adapted from Tersigni (2005).

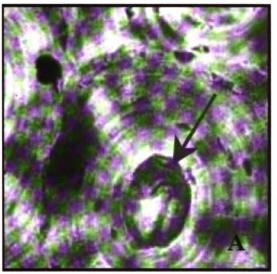
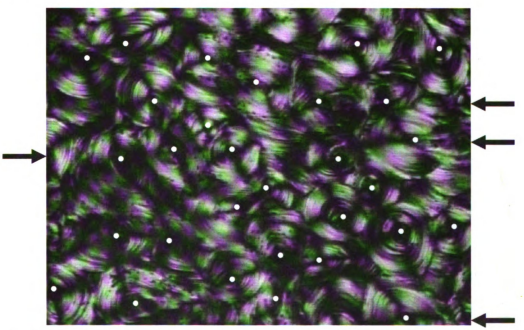


Figure 10 – Example of Hauser et al. (1980) Analysis. Circles are Identified Haversian Canals (29 present) and Arrows Indicate ½ Haversian Canals (4 present). Image Not Used in Analysis.



Appendix B

Table 3 – Pearson's r Correlation Between Real Age (RA) and Kerley-Ubelaker Age (KUA)

Correlations			
		RA	KUA
RA	Pearson Correlation	1	.652**
	Sig. (2-tailed)		.000
	N	30	30
KUA	Pearson Correlation	.652**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .05 level (2-tailed)			

Figure 11 – Graph of Correlation Between Real Age (RA) and Kerley-Ubelaker Age (KUA)

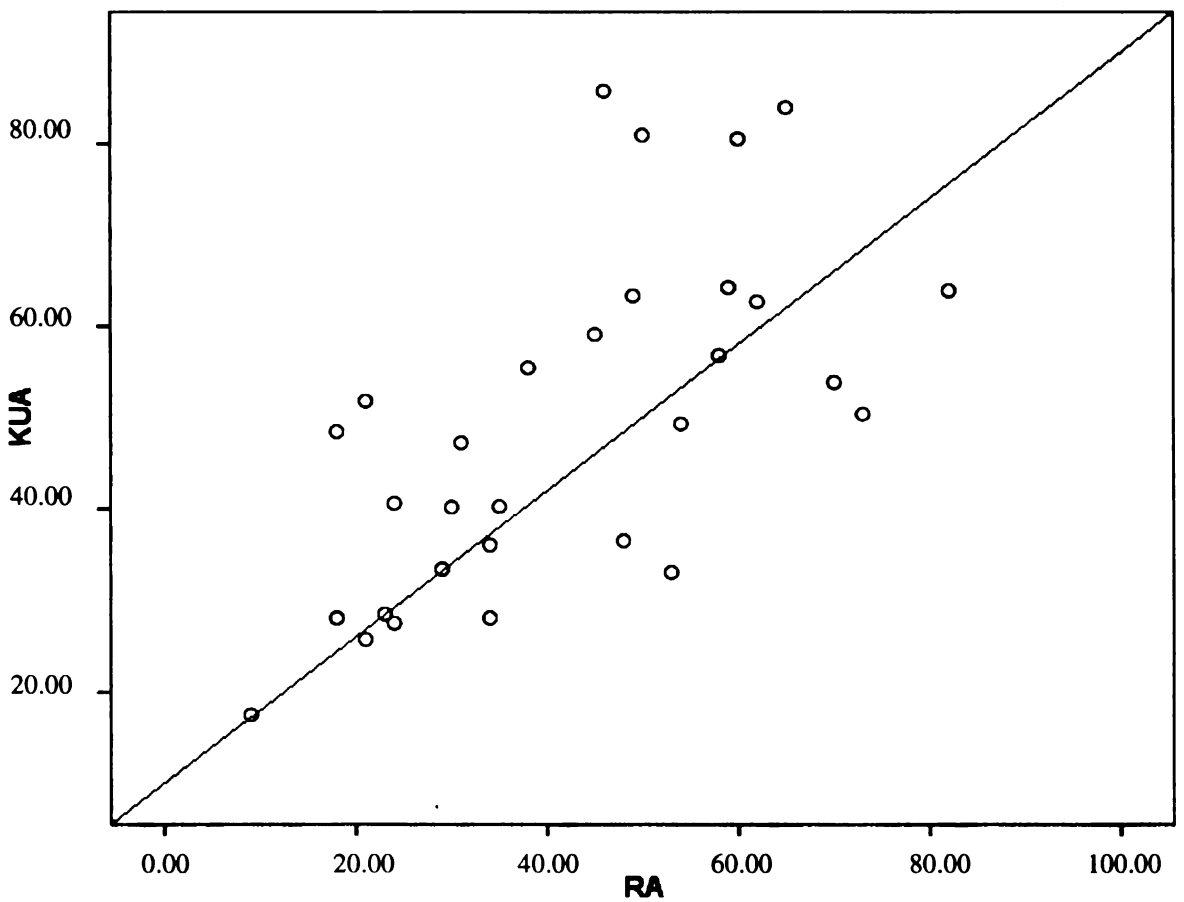


Table 4 – Pearson's r Correlation Between Real Age (RA) and Hauser-P Age (HPA)

Correlations			
		RA	HPA
RA	Pearson Correlation	1	.690**
	Sig. (2-tailed)		.000
	N	30	30
HPA	Pearson Correlation	.690**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .05 level (2-tailed)			

Figure 12 – Graph of Correlation Between Real Age (RA) and Hauser-P Age (HPA)

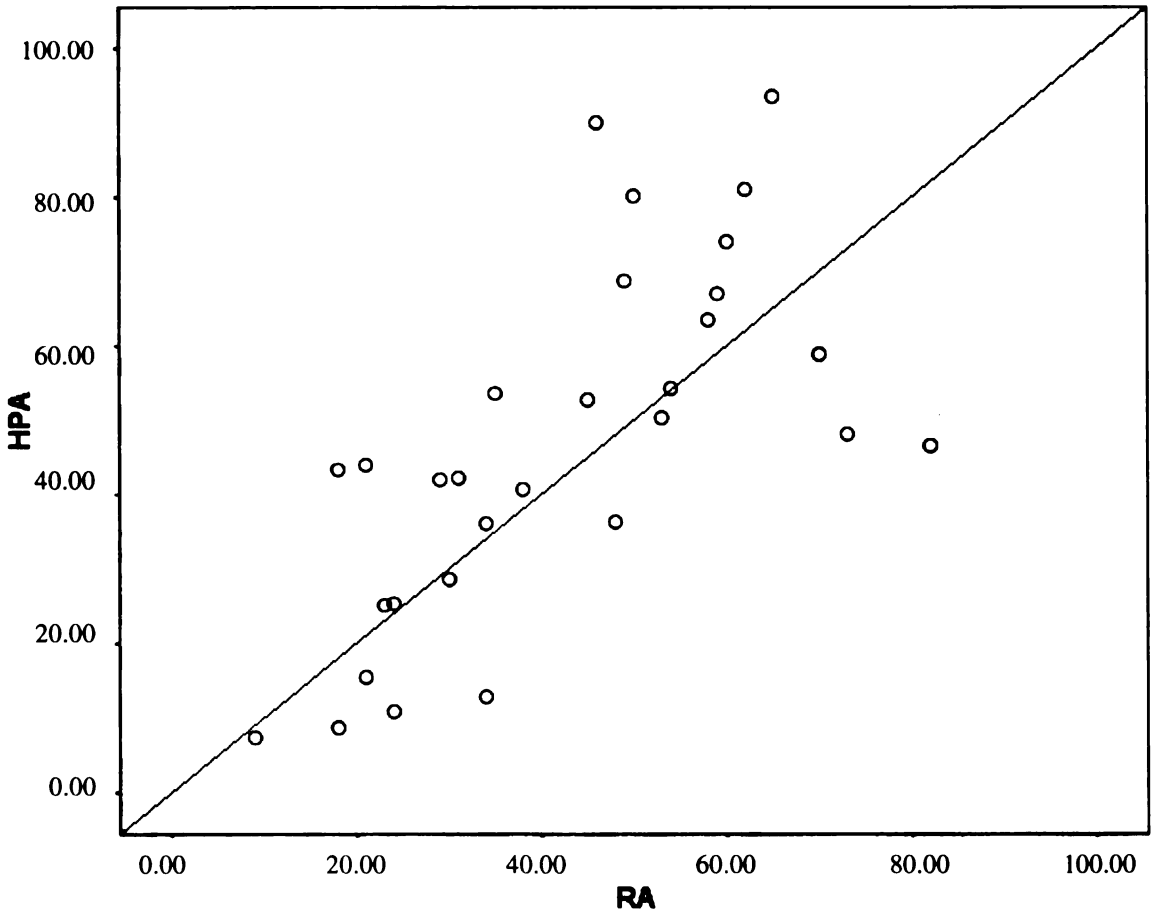


Table 5 – Pearson's r Correlation Between Real Age (RA) and Hauser-E Age (HEA)

Correlations			
		RA	HEA
RA	Pearson Correlation	1	.585**
	Sig. (2-tailed)		.000
	N	30	30
HEA	Pearson Correlation	.585**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .05 level (2-tailed)			

Figure 13 – Graph of Correlation Between Real Age (RA) and Hauser-E Age (HEA)

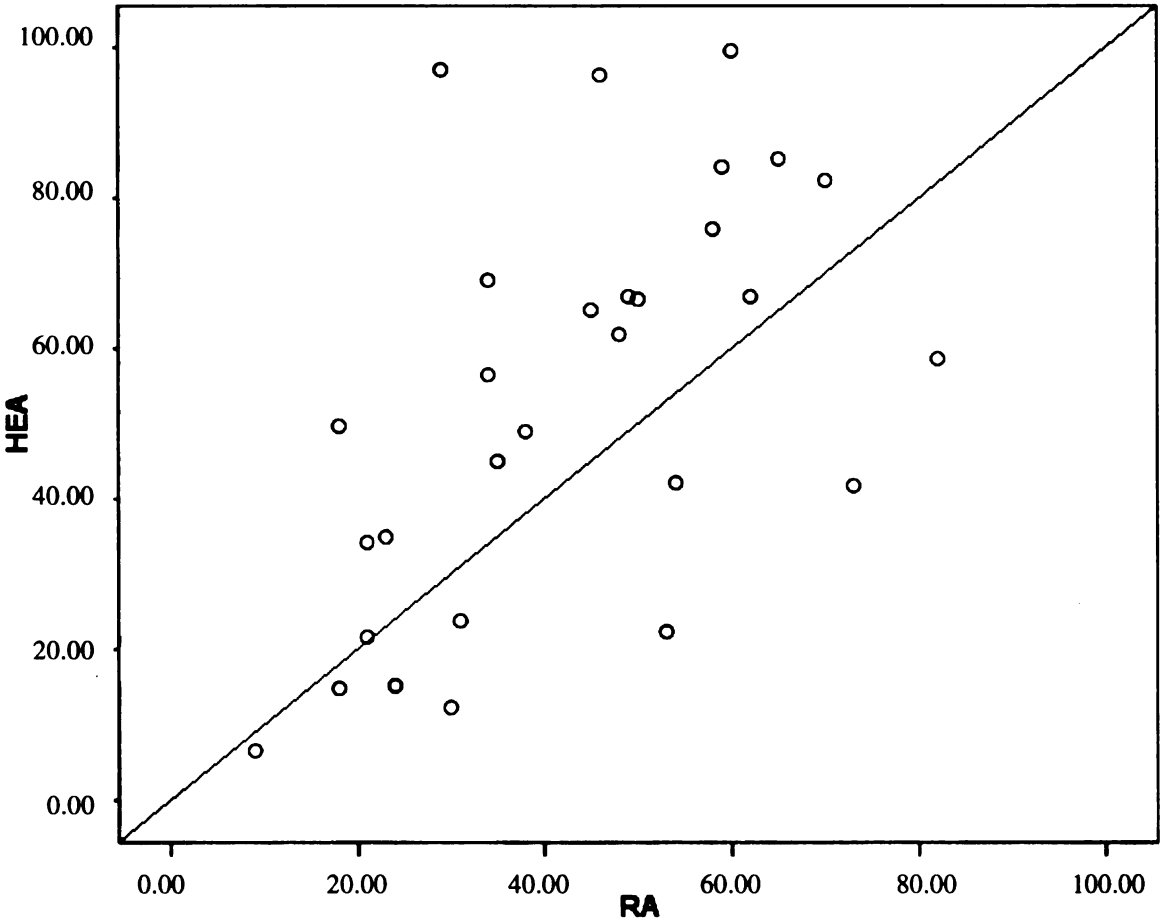


Table 6 – Pearson's r Correlation Between Real Age (RA) and Hauser-Avg Age (HAA)

Correlations			
		RA	HEA
RA	Pearson Correlation	1	.679**
	Sig. (2-tailed)		.000
	N	30	30
HAA	Pearson Correlation	.679**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .05 level (2-tailed)			

Figure 14 – Graph of Correlation Between Real Age (RA) and Hauser-Avg Age (HAA)

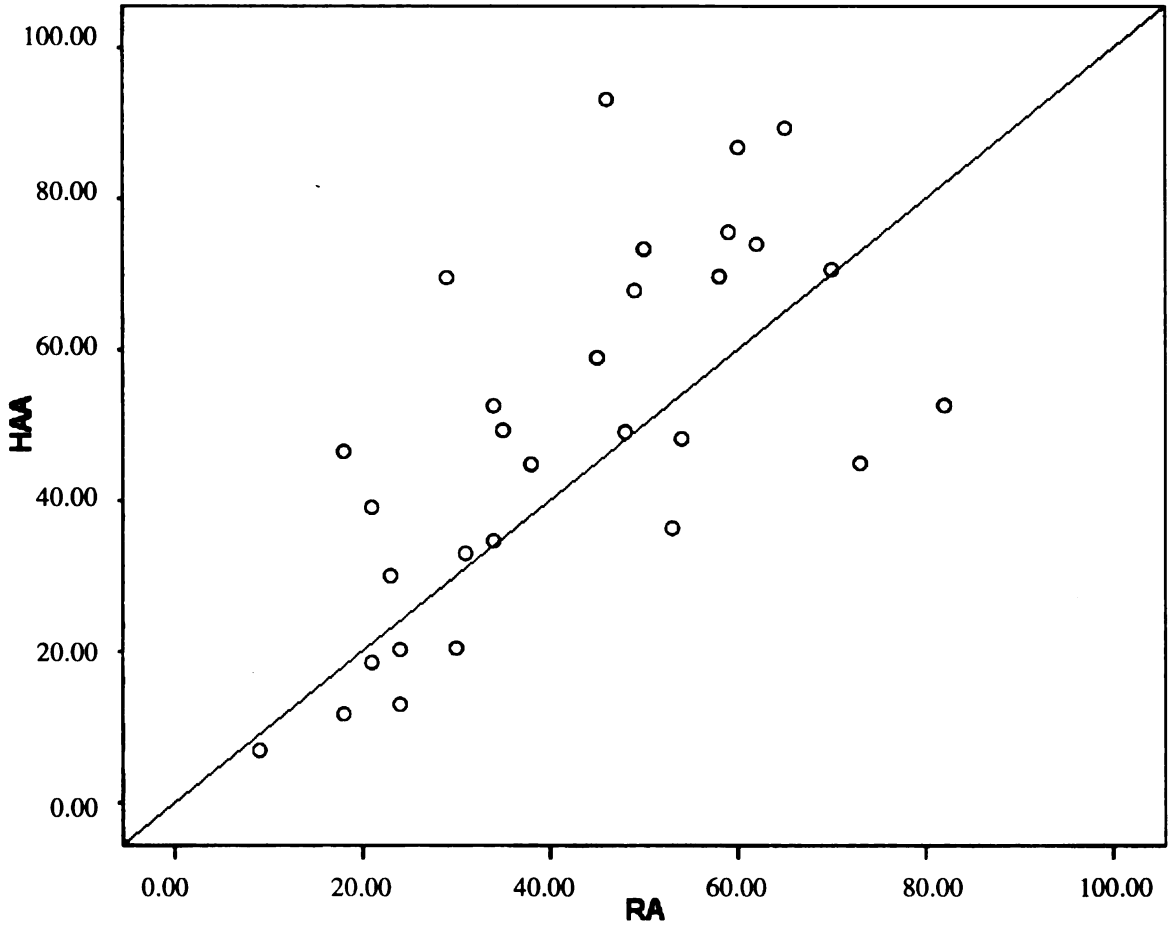


Table 7 – Paired Sample Statistics for Real Age (RA) and Kerley-Ubelaker Age (KUA), N=30

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	42.1000	30	18.88322	3.44759
KUA	49.1306	30	18.57120	3.39062

Table 8 – Paired Sample t-Test Results and Average Error (years) for Real Age (RA) and Kerley-Ubelaker Age (KUA), N=30

Paired Sample t-Test				
	df	t	Sig. (2-tailed)	Avg. Error
RA – KUA	29	-2.465	.020	13.6886
At .05 Significance level $p \leq .05$				

Table 9 – Paired Sample Statistics for Real Age (RA) and Kerley-Ubelaker Age (KUA), n=25, RA>21

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	47.0400	25	16.56170	3.31234
KUA	52.0885	25	18.01433	3.60287

Table 10 – Paired Sample t-Test Results for Real Age (RA) and Kerley-Ubelaker Age (KUA), n=25, RA>21

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
RA – KUA	24	-1.618	.119
At .05 Significance level $p \leq .05$			

Table 11 – Paired Sample Statistics for Real Age (RA) and Hauser-P Age (HPA), N=30

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	42.1000	30	18.88322	3.44759
HPA	46.7310	30	23.89787	4.36313

Table 12 – Paired Sample t-Test Results and Average Error (years) for Real Age (RA) and Hauser-P Age (HPA), N=30

Paired Sample t-Test				
	df	t	Sig. (2-tailed)	Avg. Error
RA – HPA	29	-1.453	.157	13.8116
At .05 Significance level $p \leq .05$				

Table 13 – Paired Sample Statistics for Real Age (RA) and Hauser-P Age (HPA), n=25, RA>21

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	47.0400	25	16.56170	3.31234
HPA	51.3219	25	22.40213	4.48043

Table 14 – Paired Sample t-Test Results for Real Age (RA) and Hauser-P Age (HPA), n=25, RA>21

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
RA – HPA	24	-1.193	.244
At .05 Significance level $p \leq .05$			

Table 15 – Paired Sample Statistics for Real Age (RA) and Hauser-E Age (HEA), N=30

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	42.1000	30	18.88322	3.44759
HEA	52.0254	30	27.76908	5.06992

Table 16 – Paired Sample t-Test Results and Average Error (years) for Real Age (RA) and Hauser-E Age (HEA), N=30

Paired Sample t-Test				
	df	t	Sig. (2-tailed)	Avg. Error
RA – HEA	29	-2.397	.023	19.6013
At .05 Significance level $p \leq .05$				

Table 17 – Paired Sample Statistics for Real Age (RA) and Hauser-E Age (HEA), n=25, RA>21

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	47.0400	25	16.56170	3.31234
HEA	57.3518	25	26.58337	5.31667

Table 18 – Paired Sample t-Test Results for Real Age (RA) and Hauser-E Age (HEA), n=25, RA>21

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
RA – HEA	24	-2.133	.043
At .05 Significance level $p \leq .05$			

Table 19 – Paired Sample Statistics for Real Age (RA) and Hauser-Avg Age (HAA), N=30

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	42.1000	30	18.88322	3.44759
HAA	49.3782	30	24.10868	4.40162

Table 20 – Paired Sample t-Test Results and Average Error (years) for Real Age (RA) and Hauser-Avg Age (HAA), N=30

Paired Sample t-Test				
	df	t	Sig. (2-tailed)	Avg. Error
RA – HAA	29	-2.230	.034	14.0593
At .05 Significance level $p \leq .05$				

Table 21 – Paired Sample Statistics for Real Age (RA) and Hauser-Avg Age (HAA), n=25, RA>21

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
RA	47.0400	25	16.56170	3.31234
HAA	54.3368	25	22.32866	4.46573

Table 22 – Paired Sample t-Test Results for Real Age (RA) and Hauser-Avg Age (HAA), n=25, RA>21

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
RA – HAA	24	-1.958	.062
At .05 Significance level $p \leq .05$			

Table 23 – Percentages within 5 and 10 years; 95th percentile for Kerley-Ubelaker Age (KUA), Hauser-P Age (HPA), Hauser-E Age (HEA), and Hauser-Avg Age (HAA) estimation methods/equations, N=30

Method	Percent Within 5 years	Percent Within 10 years	95 Percent Within ____
Kerley-Ubelaker Age (KUA)	23.33	46	30 years
Hauser-P Age (HPA)	26	43	30 years
Hauser-E Age (HEA)	13.33	30	50 years
Hauser-Average (HAA)	23.33	43	40 years

Table 24 – Percentages within 5 and 10 years; 95th percentile for Kerley-Ubelaker Age (KUA), Hauser-P Age (HPA), Hauser-E Age (HEA), and Hauser-Avg Age (HAA) estimation methods/equations, n = 25, RA >21

Method	Percent Within 5 years	Percent Within 10 years	95 Percent Within ____
Kerley-Ubelaker Age (KUA)	24	44	30 years
Hauser-P Age (HPA)	28	40	30 years
Hauser-E Age (HEA)	4	24	50 years
Hauser-Average (HAA)	20	40	40 years

Table 25 – Paired Differences of the Mean for Kerley-Ubelaker Age (KUA), Hauser-P Age (HPA), Hauser-E Age (HEA), and Hauser-Avg Age (HAA), N=30

Paired Differences of the Mean	
	Mean
RA – KHA	-7.0306
RA – HPA	-4.6309
RA – HEA	-9.9253
RA – HAA	-7.2781

Table 26 – Paired Differences of the Mean for Kerley-Ubelaker Age (KUA), Hauser-P Age (HPA), Hauser-E Age (HEA), and Hauser-Avg Age (HAA), n = 30, RA>21

Paired Differences of the Mean	
	Mean
RA – KHA	-5.0485
RA – HPA	-4.2818
RA – HEA	-10.3117
RA – HAA	-7.2968

Table 27 – Pearson's r Correlation Among Real Age (RA), Kerley-Ubelaker Age (KUA), Hauser-P Age (HPA), Hauser-E Age (HEA), and Hauser-Avg Age (HAA)

Correlations						
		RA	KUA	HPA	HEA	HAA
RA	Pearson Correl.	1	.652**	.690**	.585**	.679**
	Sig. (2-tailed)		.000	.000	.000	.000
	N	30	30	30	30	30
KUA	Pearson Correl.	.652**	1	.910**	.688**	.847**
	Sig. (2-tailed)	.000		.000	.000	.000
	N	30	30	30	30	30
HPA	Pearson Correl.	.690**	.910**	1	.740**	.922**
	Sig. (2-tailed)	.000	.000		.000	.000
	N	30	30	30	30	30
HEA	Pearson Correl.	.585**	.688**	.740**	1	.943**
	Sig. (2-tailed)	.000	.000	.000		.000
	N	30	30	30	30	30
HAA	Pearson Correl.	.679**	.847**	.922**	.943**	1
	Sig. (2-tailed)	.000	.000	.000	.000	
	N	30	30	30	30	30
**Correlation is significant at the .05 level (2-tailed)						

Table 28 – Paired Sample Statistics for Kerley-Ubelaker Age (KUA) and Hauser-P Age (HPA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
KUA	49.1306	30	18.57120	3.39062
HPA	46.7310	30	23.89787	4.36313

Table 29 – Paired Sample t-Test Results for Kerley-Ubelaker Age (KUA) and Hauser-P Age (HPA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
KUA – HPA	29	1.261	.217
At .05 Significance level $p \leq .05$			

Table 30 – Paired Sample Statistics for Kerley-Ubelaker Age (KUA) and Hauser-E Age (HEA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
KUA	49.1306	30	18.57120	3.39062
HEA	52.0254	30	27.76908	5.06992

Table 31 – Paired Sample t-Test Results for Kerley-Ubelaker Age (KUA) and Hauser-E Age (HEA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
KUA – HEA	29	-.787	.438
At .05 Significance level $p \leq .05$			

Table 32 – Paired Sample Statistics for Kerley-Ubelaker Age (KUA) and Hauser-Avg Age (HAA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
KUA	49.1306	30	18.57120	3.39062
HAA	49.3782	30	24.10868	4.40162

Table 33 – Paired Sample t-Test Results for Kerley-Ubelaker Age (KUA) and Hauser-Avg Age (HAA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
KUA – HAA	29	-.105	.917
At .05 Significance level $p \leq .05$			

Table 34 – Paired Sample Statistics for Hauser-P Age (HPA) and Hauser-E Age (HEA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
HPA	46.7310	30	23.89787	4.36313
HEA	52.0254	30	27.76908	5.06992

Table 35 – Paired Sample t-Test Results for Hauser-P Age (HPA) and Hauser-E Age (HEA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
HPA – HEA	29	-1.529	.137
At .05 Significance level $p \leq .05$			

Table 36 – Paired Sample Statistics for Hauser-P Age (HPA) and Hauser-Avg Age (HAA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
HPA	46.7310	30	23.89787	4.36313
HAA	49.3782	30	24.10868	4.40162

Table 37 – Paired Sample t-Test Results for Hauser-P Age (HPA) and Hauser-Avg Age (HAA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
HPA – HAA	29	-1.529	.137
At .05 Significance level $p \leq .05$			

Table 38 – Paired Sample Statistics for Hauser-E Age (HEA) and Hauser-Avg Age (HAA)

Paired Sample Statistics				
	Mean	N	Std. Deviation	Std. Error Mean
HEA	52.0254	30	27.76908	5.06992
HAA	49.3782	30	24.10868	4.40162

Table 39 – Paired Sample t-Test Results for Hauser-E Age (HEA) and Hauser-Avg Age (HAA)

Paired Sample t-Test			
	df	t	Sig. (2-tailed)
HEA – HAA	29	1.529	.137
At .05 Significance level $p \leq .05$			

Table 40 – Pearson's r Correlation Between Kerley-Ubelaker Age 1 (KUA1) and Kerley-Ubelaker Age 2 (KUA2)

Correlations			
		KUA1	KUA2
KUA1	Pearson Correlation	1	.907**
	Sig. (2-tailed)		.000
	N	30	30
KUA2	Pearson Correlation	.907**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .01 level (2-tailed)			

Table 41 – Pearson's r Correlation Between Hauser-P Age 1 (HPA1) and Hauser-P Age 2 (HPA2)

Correlations			
		HPA1	HPA2
HPA1	Pearson Correlation	1	.926**
	Sig. (2-tailed)		.000
	N	30	30
HPA2	Pearson Correlation	.926**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .01 level (2-tailed)			

Table 42 – Pearson's r Correlation Between Hauser-E Age 1 (HEA1) and Hauser-E Age 2 (HEA2)

Correlations			
		HEA1	HEA2
HEA1	Pearson Correlation	1	.864**
	Sig. (2-tailed)		.000
	N	30	30
HEA2	Pearson Correlation	.864**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .01 level (2-tailed)			

Table 43 – Pearson's r Correlation Between Hauser-A Age 1 (HAA1) and Hauser-A Age 2 (HAA2)

Correlations			
		HAA1	HAA2
HAA1	Pearson Correlation	1	.913**
	Sig. (2-tailed)		.000
	N	30	30
HAA2	Pearson Correlation	.913**	1
	Sig. (2-tailed)	.000	
	N	30	30
**Correlation is significant at the .01 level (2-tailed)			

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