HISTOMORPHOMETRY OF THE HUMAN RIB CORTEX IN METHAMPHETAMINE USERS

by

Robert Chancey Karinen

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Arts in Anthropology

Boise State University

December 2009

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Robert Chancey Karinen

Thesis Title: Histomorphometry of the human rib cortex in methamphetamine users

Date of Final Oral Examination: 14 October 2009

The following individuals read and discussed the thesis submitted by student Robert Chancey Karinen, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Margaret Streeter, Ph.D.	Chair, Supervisory Committee
Mark Plew, Ph.D.	Member, Supervisory Committee
Christopher Hill, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Margaret Streeter, Ph.D., Chair of the Supervisory Committee. The thesis was approved for the Graduate College by John R. Pelton, Ph.D., Dean of the Graduate College.

ACKNOWLEDGEMENTS

Dr. Margaret Streeter- for serving as my advisor and chair of my committee. For introducing me to bone histology. For the countless hours of advice and education. I was truly lucky to have a advisor that is whole heartedly committed to the education of her students.

Dr. Mark Plew- for serving on my committee and providing support and encouragement throughout my graduate education.

Dr. Christopher Hill- for serving on my committee. The education in statistics provided by you is what gives my research meaning.

Ada County Coroners Office- For keeping me employed during this process and allowing me a source from which to collect data.

To my fellow students from which I have learned from and who have made this experience all the more rewarding.

To my wife Jenny who has always supported me in whatever endeavor I undertake. Thank you for everything you do for our family. I look forward to being able to return to our life together.

To the decedents included in the study.

ABSTRACT

Studies have demonstrated lifestyle choices such as poor diet, insufficient exercise and substance abuse can negatively affect bone health. The purpose of this research is to determine if the tissue pathology associated with long term methamphetamine use is a localized response to poor dental hygiene or an indication of a more systemic response that is discernable in the bone microstructure. A comparison of the rib cortical bone microstructure between males that were known to be methamphetamine abusers (N=18) and individuals who did not abuse the drug was undertaken (N=19). Histomorphometric variables calculated in this analysis included mean osteon size, osteon population density (OPD) and cortical area measurement. OPD was found to vary significantly between study and control populations. This study demonstrates that methamphetamine abuse is associated with changes in OPD.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF CHARTS AND GRAPHS

CHAPTER 1: INTRODUCTION

Bone is a dynamic tissue that is capable of responding to metabolic stress (Frost, 1963; Enlow, 1976). Studies have demonstrated lifestyle choices such as poor diet, insufficient exercise and substance abuse can negatively affect bone health (Peris et al., 1992; Hernandez-Avila, Stampfer and Ravnikar, 1993; Schnitzler 1993; Felson, Zhang, Hannan, Kannel & Kiel, 1995). Habitual users of methamphetamines exhibit marked deterioration of their dentition and associated oral and facial soft tissue (Shaner, Kimmes, Saini & Edwards, 2006; Spalding 2006; Cadet, Jayanthi and Deng, 2003; Padilla and Ritter 2008; Heng, Badner and Schiop, 2008, McKetin, Kelly, McLaren and Proudfoot, 2008; Hamamoto and Rhodus 2009). Studies have associated chronic methamphetamine abuse with lower bone densities in both the axial and appendicular skeleton (Katsuragawa, 1999; Kim et al., 2009) but, no studies examine how methamphetamine may be impacting the microscopic structure of bone. The purpose of this analysis is to determine if connective tissue pathology associated with long term methamphetamine use is discernable in the cortical bone microstructure of the fourth rib. This study will evaluate three microscopic observations. These are cortical area, mean osteon size, and osteon population density.

Chapter two will provide a background on some basic concepts of bone histology. An explanation of each of the study variables will then be undertaken. This will be followed by an examination of the different factors known to influence bone

microstructure. Included in this discussion will be a detailed look at the role that methamphetamine plays in the human body.

In chapter three the materials and methods used in this study will be described in detail. This will be followed by chapter four in which a comparison of the rib cortical bone microstructure between white males that were known to be methamphetamine abusers (N=18) and individuals who had no known history of drug abuse (N=19). This study will test three histomorphometric variables using the point count method. These are mean osteon size, osteon population density (OPD) and cortical area measurements. Each of these variables will be tested independently in an effort to explore how methamphetamine effects bone microstructure. The results of this analysis will then be described in chapter five.

In chapter six conclusions of this study will be described as well as some suggestions for future avenues of study. The conclusions reached in this study have important applications for physical anthropology. Methamphetamine users have been shown to have an increased risk of homicidal, suicidal or accidental death (Logan, Fligner and Haddix, 1998; Zweben et al., 2004). Whether from intentional hiding of homicide victims, or remoteness of death location, significant time may pass between death and discovery. When limited, or taphonomically damaged remains are recovered histological age estimations may be employed in order to assist in identification. Histological age estimations are dependent on observations of bone microstructure. These estimations will not be accurate if the unidentified individual has a history of methamphetamine abuse. This study also supports previous publications that bone health is negatively affected by methamphetamine use (Katsuragawa, 1999; Kim et al., 2009). In

addition it adds to a growing body of evidence that bone microstructure is sensitive to a multitude of variables that can skew histological analysis (Peris et al., 1992; Hernandez-Avila et al., 1993; Schnitzler, 1993; Felson et al., 1995).

CHAPTER 2: BACKGROUND

Bone Composition

Bone is a composite tissue that is made up of both organic and inorganic components. It is important to recognize that this unique blend of organic and inorganic molecules is what gives bone its ability to support the body but remain responsive enough to absorb enormous stresses associated with life (Tortora and Grabowski, 1996). Bone consists of water (25%), organic matrix (32%), minerals (42%) and organic proteins (1%) such as osteocalcin (Martin, Burr and Sharkey, 1998). Ninety percent of the organic portion of bone is made up of the large protein molecule collagen. Collagen, the most common protein in the body, forms molecules that combine to form flexible elastic fibers. Collagen provides the skeleton with flexibility and elasticity. In mature bones collagen is stiffened by the mineral compound hydroxyapatite (Martin et al., 1998; Tortora and Grabowski, 1996). Hydroxyapatite ($Ca_5(PO_4)_4(OH)_2$ takes the form of crystals which are imbedded within the collagen matrix. This is what gives bone its rigidity and compressive strength.

Functions of Bone

Bone carries out numerous important functions within the body. These include mechanical functions such as supporting and protecting internal organs and providing a framework from which tendons and ligaments can attach. Bone also serves numerous

physiological functions. These include being centers for hemopoiesis, storage facilities for fat and as reservoirs for important elements such as calcium (Martin et al., 1998).

Bone Cells

Bone is made up of several cell types. These are osteoblasts, bone lining cells, osteocytes and osteoclasts. Osteoblasts create and deposit new bone material. They do this by creating osteoid (unmineralized bone) which is a collagen rich uncalcified organic matrix. Calcification occurs when crystallized hydroxyapatite is deposited onto the matrix. During this process some osteoblasts become trapped within the bony matrix. When this occurs the osteoblast becomes an osteocyte (Parfitt, 1988).

Bone lining cells are former osteoblasts that flatten out and become inactive but are still able to reactivate as osteoblasts when needed (Martin et al., 1998). These cells are present under the periosteum as well lining the Haversian and Volksman canals (Parfitt, 1988). Bone lining cells are similar to osteocytes in that they are involved in mineral homeostasis and are part of the sensory network of osteocytes. However, they differ from osteocytes because of their ability to reactivate as osteoblasts and commence bone synthesis (Klein-Nulend et al., 1995; Martin et al., 1998; Metz, Martin and Turner, 2003; Parfitt, 1996). Osteocytes reside within the bony tissue and are responsible for maintaining bone metabolism (Parfitt, 1988; White, 2005). The space in which they reside is referred to as the osteocytic lacunae. Each osteocyte is connected within a network of canaliculi. These canaliculi eventually link the osteocytes to the circulatory system from which they receive the products necessary to maintain bone health and respiration (Tortora and Grabowski, 1996). These vascular structures are known as Haversian and Volksman's canals. Haversian canals run the length of the bone with

Volksman's canal running off at oblique and right angles (Frost, 1963; Marks and Popoff, 1988).

Osteoclasts are large multi-nucleinated cells that resorb bone. Osetoclasts work in conjunction with osteoblasts to constantly maintain and reshape bones during growth and development. These actions are referred to as modeling when discussing the sculpting of bone during growth and remodeling when referring to the regeneration of damaged bone. (Martin et al, 1998; White, 2000). A more detailed discussion of modeling and remodeling can be found in a later section.

Bone Mass and Bone Density

Bone mass is a measure of the quantity of bone or refers to the area of mineralized bone. This differs from bone density which is a measure of bone unit volume. Bone density not only measures the amount of mineralized cortex but also takes into account void spaces in the marrow cavity and intracortical porosity (Frost, 1963; Martin et al., 1998). Bone density and bone mass both play a role in the risk of fracture.

Bone Growth, Modeling and Remodeling

Modeling and Growth

The development of a skeleton into its adult form is the result of the unified actions of both growth and modeling. In long bones growth acts to increase both bone length and diameter as directed by the individual's genetic plan (Robling and Stout, 2008). Modeling works to sculpt the bone into a shape that is most mechanically stable for the stresses being placed on the organism during life. Modeling involves the resorption or formation of bone at a single locus (Robling and Stout, 2008). In other words osteoblasts and osteoclasts act independently at different sites during the modeling

process. The law of bone transformation states that bone is laid down where it is needed and resorbed where it is not needed (Martin et al, 1998). Throughout life the shape of a bone is determined by the biomechanical forces that act upon it.

How bone responds to mechanical stress during maturation results in a process described as modeling drifts. Stress, or lack of stress, causes bone to either be added by osteoblasts or taken away by osteoclasts. This shaping of bone occurs on the periosteal and endosteal surfaces. By adjusting the rate at which bone is resorbed and laid down these modeling drifts effectively move bone through tissue space (Scheuer and Black, 2004; Robling and Stout, 2008). As a result of this drift bone laid down early in the process eventually is removed or is modeled out. Because of these processes lamellar bone in an adult skeleton may contain bone of varying ages. However, the average tissue age is always lower than the chronological age because of the nature of the drifts (Robling and Stout, 2008). Bone created by osteoblasts during modeling is lamellar bone that differs microscopically from bone which is remodeled (Enlow, 1960; Parfitt, 1988; Martin et al., 1998). Modeled bone appears as parallel sheets of primary lamellar bone while remodeled bone appears as concentric packets within osteons. In most cases modeling slows to a negligible rate once skeletal maturity is reached (Frost, 1969). Modeling can be reactivated in adults under certain circumstances such as injury, disease or when bones are placed under mechanical loads (Martin et al., 1998).

Remodeling

Remodeling is the process by which the body renews bone. Remodeling is also the mechanism for altering bone structure in the adult skeleton (Martin et al., 1989). Remodeling starts before birth and continues throughout life. Bone remodeling always

follows activation, resorption and formation sequence at a particular locus (Robling and Stout, 2008) Remodeling differs from modeling in that it occurs throughout life (Frost, 1963). Bone remodeling is a physiological response to damage caused through mechanical stresses and aging (Martin et al., 1998). There is also a degree of base line remodeling that is not associated with repair of damage (Frost, 1987a). The agent responsible for remodeling is known as the basic multicellular unit or BMU. This is explained in more detail below.

The rate of osteon production is bone specific (Enlow, 1976; Peck and Stout, 2006). Increasing the stress placed on bone results in increased microscopic damage. Weight bearing bones such as the femur and tibia are examples of bones that suffer from an increase of such stress. This will cause certain bones to remodel at a faster pace than bones undergoing less biomechanical stress. Ribs have also been shown to have relatively accelerated turnover rates (Stout, 1995). The rate of osteon production also varies depending on the location within the bone (Frost, 1963). The parts of bones that undergo the most mechanical stress are most likely to suffer microdamage. It is these damaged areas that will initiate the bone remodeling unit in an effort to repair damage from stress. Osteon production can also be affected by other natural processes such as age, sex, health status and physical activity (Martin et al., 1998). It is important that such factors be taken into account in order to accurately distinguish between normal and abnormal rates of remodeling.

The Basic Multicellular Unit

The basic mulitcellular unit (Figure 2.1) is a complex arrangement of cells that is responsible for the formation of osteons. The BMU consists of a cutting cone and a closing cone. The BMU travels in a longitudinal direction within the cortex of long bones (Frost, 1963; Parfitt, 1988; Hert, Fiala and Petrtyl, 1994). As the BMU moves through the cortical bone the leading edge is lined with osteoclasts. These osteoclasts resorb bone creating a void within the cortical space. When viewed in cross section this void is called a Howships's lacunae and is typically between 250 and 300 micrometers across its maximum with rough scalloped edges (Robling and Stout, 2008). Behind the osteoclasts a group of mononuclear cells follows. The exact purpose of these cells remains unclear (Robling and Stout, 2008). However it has been hypothesized that the mononuclear cells may prepare the scalloped edges of the resorptive bay as a conducive surface for the reversal line (Robling and Stout, 2008).

The reversal line is a thin mineral rich layer of matrix that separates the osteon from the surrounding matrix (Martin et al., 1998; Schaffler, Burr and Frederickson, 1987). Following the mononuclear cells are rows of osteoblasts that deposit unmineralized bone material called osteoid centripetally to the surfaces of the resporptive bay (Robling and Stout, 2008). This layering continues and forms concentric osteonal lamellae that become mineralized. This action slowly decreases the diameter of the void until it ceases leaving an Haversian canal in the center.

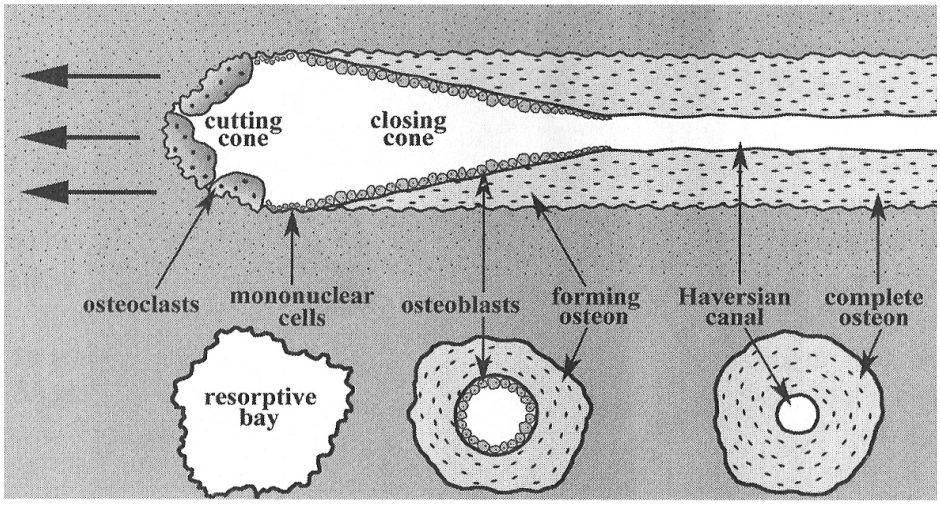


Figure 2.1 Longitudinal View of the BMU

Robling and Stout, 2008

Growth of Ribs

Ribs begin growing endochondrally at their primary centers of ossification during the second intrauterine month (Scheuer and Black, 2004). By birth the ribs have obtained the basic morphology found in adults. However, the ribcage in an infant is oriented horizontally and in early childhood the torsion of the shaft causes the ventral portion of the ribs to drift downward as in the adult thorax. This is a reflection of the diaphragmatic involvement in breathing and a more active role of the rest of the thorax in respiration (Scheuer and Black, 2004). During the growth associated with childhood the rib cage is expanding to adult proportions via cortical drift (Figure 2.2). Cortical drift moves the cortex away from the pleural cavity and out toward the cortical surface (Enlow, 1960; Frost, 1963; Epker and Frost, 1965). Due to this mechanism older bone is resorbed on the plural-periosteal surface and the cutaneous- endosteal surfaces while bone is deposited on the pleural- endosteal and cutaneous-periosteal surface of the ribs (Landeros and Frost, 1966).

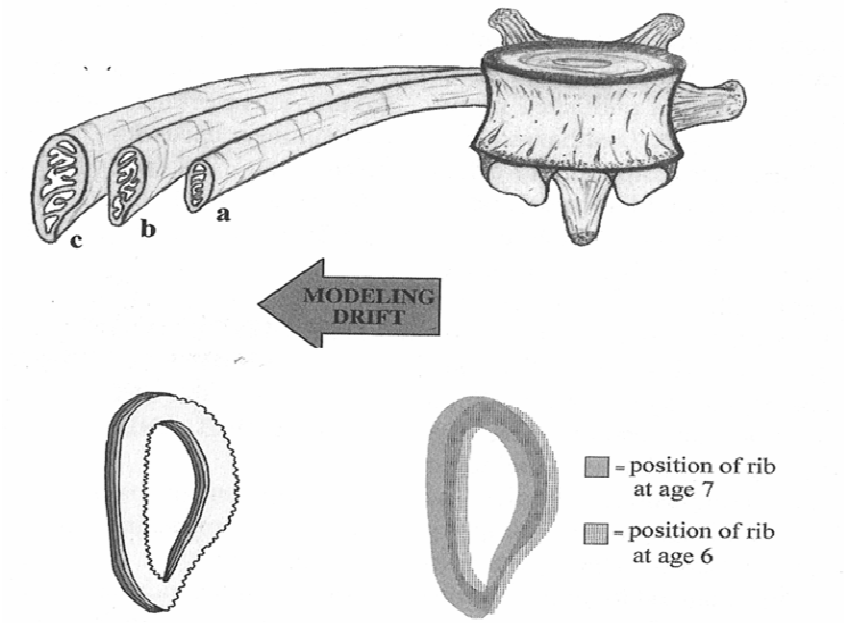


Figure 2.2 Modeling Drift of Human Rib (Robling and Stout 2008)

Anatomy of a Rib

All bones including the ribs are covered with a thin layer of tissue known as the periosteum. The periosteum is connected to the bone by collagenous fibers known as Sharpey's fibers. The periosteum itself is made up of two parts, a fibourous layer and an osteogenic layer. Within the fibrous layer nerve tissue and blood vessels are located. It is also where muscles attach themselves to the bone. The osteogenic layer lies next to the bone and contain progenitor cells that have the ability to develop into osteoblasts (Tortora and Grabowski, 1996). The inner surface of the medullary cavity is lined with another fibrous membrane known as the endosteum (Figure 2.3).

Ribs are made up of both cortical and trabecular bone. Cortical or compact bone is densely organized and gives the rib its overall shape and structure. Within the medullary cavity lies spongy or trabecular bone (Figure 2.3). Trabecular bone has a much

greater surface area than cortical bone and is highly vascular. It is within the trabecular bone that bone marrow lies and hematopoesis or red blood cell production occurs (Tortora and Grabowski, 1996). Trabecular bone is less dense and soft when compared to cortical bone.

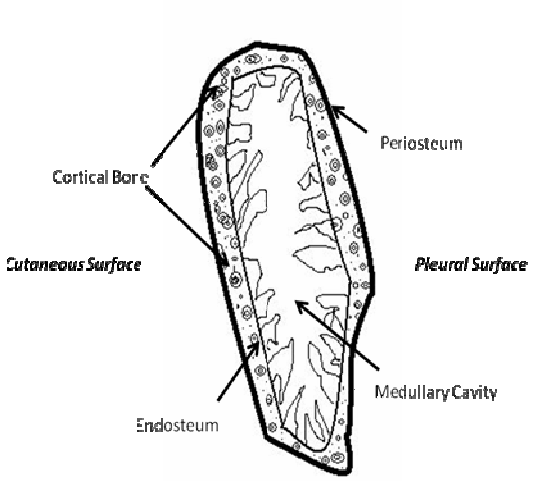


Figure 2.3 Cross Section of Rib

Interstitial Bone and Osteons

Interstial bone lies between osteons and makes up the unremodeled cortex. (White, 2000). Interstitial bone is composed of lamellar bone including fragments of osteons. Osteon fragments are former osteons that have been partially obliterated during the formation a new osteon. The relative area of interstitial bone is inversely related to age decreasing over time as it is gradually replaced by osteons (Frost, 1963; Parfitt, 1988).

Four distinct types of secondary osteons have been identified. These include Type I, Type II, drifting and zonal varieties. While these are identified as being different types

all osteons are the product of the same actions of the basic multicellular unit (Robling and Stout, 2008).

Type I osteons are the most common type seen in adult humans (Takahashi and Frost, 1965). Type I osteons are known as secondary osteons. They consist of circular concentric layers of lamellar bone surrounding a Haversian canal (Martin et al., 1998). When viewed under the microscope in cross section these osteons appear as discrete circular packages of concentric bone that are bordered by a scalloped reversal or cement line (Martin et al., 1998). Type I osteons result from normal intracortical remodeling (Martin et al., 1998).

Type II osteons are also called embedded osteons. They result from remodeling along a length of Haversian canal within a previously existing osteon (Ericksen, 1991; Richman et al., 1979). When viewed in cross section type II osteons appear as an osteon within an osteon (Cohen and Harris, 1958; Jaworski, Meunier and Frost, 1972; Ortner, 1975; Parfitt, 1983) and exhibit two scalloped reversal lines one within the other (Frost, 1963; Takahashi and Frost, 1966). Type II osteons are thought to be correlated with non-specific stress (Ericksen, 1991; Frost, 1963; Ortner, 1975; Richman et al., 1979; Stout and Simmons, 1979; Takahashi and Frost, 1966). Stout and Simmons (1979) found that type II osteons could be linked to times of dietary stress. Because of this the presence of type II osteons can be used as evidence of a disturbance to normal intracortical osteon production. Some research has suggested that the density of type II osteons is positively correlated with age (Yoshino, Imaizumi, Miyasaka and Seta, 1994; Ericksen, 1991). However Richman et al.'s (1979) study of aboriginal Americans suggested that there is no change in the quantity of secondary osteons over time.

Drifting or waltzing osteons look similar to type I osteons except they appear elongated rather than circular when observed under the microscope. They are described as having a hemicyclic lamellar tail (Robling and Stout, 1999). They also have eccentric haversian canals (Frost, 1963; Sedlin, Frost and Villanueva, 1963; Epker and Frost, 1965; Coutelier, 1976; Burton, Nyssenn-Behets and Dhem, 1989; Frost, 1987b; Robling and Stout, 1999). Drifting osteons are the most common type of osteon found in sub adults (Burton et al., 1989; Streeter 2005).

Like type II osteons, zonal osteons are also the result of a disturbance of normal intracortical osteon production (Martin et al., 1998). They are created during the infilling stage of a Type I osteon. In cross section they appear similar to type II osteons in that they look like a small osteon within an osteon. However they differ by containing one or more smooth arrest line as well as parallel contours of concentric lamellae (Frost; 1963; Stout and Simmons, 1979). In zonal osteons there is a disturbance in radial closure during the creation of a new type I osteon (Pankovich, Simmons and Kulkarni, 1974; Parfitt, 1983). This disturbance can be seen in a microradiograph as a hypercalcified ring within the lamellae which is denser than surrounding lamellar bone. This ring results from a pause in bone deposition that is ongoing for at least a month (Frost, 1963; Parfitt, 1983; Stout and Simmons, 1979).

Zonal osteons have been associated with disease and aging (Pankovich et al., 1974). For example zonal osteons have been associated with the macroscopic indicator of stress Harris lines (Stout and Simmons, 1979). Zonal osteons have also been found to increase with age at a rate of approximately 4% per decade in individuals between 20 and 80 years of age (Pankovich et al., 1974).

There has been some effort to associate increasing densities of double zonal osteons to age but it has met with mixed results. Yoshino found that such double zonal osteons decrease with age in his study of the humerus (Yoshino et al., 1994). This study contradicts Pankovich's study of the rib which found zonal osteons to be positively correlated (Pankovich et al., 1974). Stout and Simmons also looked at the humerus but found no relationship between zonal osteons and age (Stout and Simmons, 1979).

The distributions of osteons within a given cross section of rib do not follow a consistently dispersed pattern despite the fact that remodeling can occur on any bone surface not covered by cartilage or osteoid (Martin et al., 1998). Osteons are more likely to appear in areas in response to areas of damage caused by biomechanical forces. For example, when bones experience increased mechanical loading there is an increase in osteon production (Burr, Martin, Schaffler and Radin, 1985; O'Conner, Lanyon and MacFie, 1982; Raab, Crenshaw, Kimmel and Smith, 1991). Osteons are also intricately linked to Haversian systems and membrane surfaces tending to cluster in these areas (Enlow, 1960; Parfitt, 1988; Martin & Burr, 1982; Tappen, 1977). This occurs because osteoblasts are hemopoetic in origin (Rodan and Martin, 1981)

Cortical Area

In cross section the area occupied between the subperisoteal area and endosteum is made up of cortical bone. The meduallry cavity contained within the endosteum is occupied by trabecular bone and is not used in this measurement (Figure 2.3). Cortical area is a measure of the amount of cortical bone within a representative cross section between the periosteal and endosteal layers. The amount of cortical area follows a pattern of increasing from birth through the attainment of peak bone mass (Sedlin et al., 1963).

Once peak bone mass is reached cortical area slowly decreases throughout life due to expansion of the marrow cavity (Sedlin et al., 1963).

Mean Osteon Size

Mean osteon size is the average area of bone including Haversian Canals contained within the cement lines of a structurally intact osteon. Throughout the body mean osteon size varies from bone to bone ranging from .02 mm^2 to .07 mm^2 (Qiu, Fyhrie, Palnitkar and Rao, 2003). For instance it has been shown that mean osteon size is greater in the femur when compared to the tibia (Kerley, 1965; Martin et al., 1998). Mean osteon size within an adult human rib is .037 mm^2 (Wu, Shubeck, Frost and Villanueva, 1970).

Some studies have suggested that osteon size decreases with age (Yoshino et al., 1994; Jowsey, 1966; Currey, 1964). However other studies have found that there is no apparent change in the size of the osteon over time (Jowsey, 1968). The relationship that sex plays in mean osteon size is also unclear. One study suggests that osteons were found to increase in size over time in females whereas in males they decrease (Burr, Ruff and Thompson, 1990). This is in contrast to an earlier study in which the exact opposite was found (Broulik, Kragstrup, Moskilde and Melsen, 1982). A recent studies based on 20^{th} century populations have suggested that there is no difference between sexes (Pfeiffer, 1998). Mean osteon size can be an indicator of overall cell activity Smaller osteon size is associated with less vigorous osteoclast activity (Martin et al., 1998).

Osteon Population Density

As an individual ages osteon density increases on the cortical surface. Osteon population density (OPD) is a measure of the number of whole and fragmentary osteons per mm^2. Numerous studies have demonstrated that OPD is positively associated with age (Kerley, 1965; Ahlqvist and Damsten, 1969; Singh and Gunberg, 1970; Iwamoto, Oonuki and Konishi, 1978; Rother, Kruger, Mechlitt and Hunger, 1978; Erickson, 1991; Stout and Paine, 1992; Kimura, 1992; Uytterschaut, 1985; Yoshino et al., 1994; Cool, Hendrikz and Wood, 1995: Stout and Lueck, 1995: Stout and Paine, 1996). Because of this relationship OPD has been employed in the determination of age at death from histological material (Thompson, 1979; Hauser, Barres, Durigon & Delbert, 1980; Fangwu, 1983; Thompson and Galvin, 1983; Drusini, 1996; Uytterschaut, 1985; Samson and Branigan, 1987; Drusini, 1987; Drunini and Businaro, 1990; Narasaki, 1990; Stout and Paine, 1992) These estimates have proven most accurate when analyzing individuals between the second and fifths decades of life. This method continues to be useful until the entire cortical surface has been filled with osteons signaling asymptote during the fifth or sixth decade of life. At this point any new osteons will be completely obliterating any evidence of previous osteon fragments. It is also at this point that OPD no longer can be correlated with age.

There are some challenges to using osteon counts for histological age estimation. The first is how to determine what constitutes a secondary osteon. Various studies have suggested inconsistent criteria for what exactly constitutes a secondary osteon (Kerley, 1965; Wu et al. 1970; Ortner, 1975; Ericksen, 1991). Osteon population's density within the cortex has been shown to vary within not only an individual bone but between

different bones of one individual (Frost, 1969; Jaworski et al., 1972; Stout and Gehlert, 1980; Stout and Lueck., 1995; Pfeiffer, 1998; Walker 1996).

Histological Estimations of Age

Bone is a responsive tissue that has physiological plasticity when it comes to growth and repair. It is also recognized that bones are dynamic structures that undergo complex biological processes that have the potential to be disrupted at any number of steps. The physiological record of these processes is preserved in the macroscopic and microscopic anatomy of bones and allows skeletal biologists to interpret these metabolic processes and employ them in the analysis of developmental and behavior patterns of past and recent population (Streeter, 2007). The increasing knowledge of how bones respond to physiological stress has allowed regression formulas to be developed that can be used to estimate age at death. The first age predicting equation based on histological observations was conducted by Balthazard and Lebron in 1911. However age estimation techniques did not begin to be widely used until Kerley published his method of estimations on the femur, tibia and fibula (Kerley, 1965; Kerley and Ubelaker, 1978).

Age dependent variables that have been associated with increasing age include cortical area and osteon density. Some studies have suggested using mean osteon size as an age dependent variable though it remains controversial (Yoshino et al., 1994; Currey, 1964; Jowsey, 1966; Pfeiffer, 1998).The association of OPD to aging is well documented and several age estimation formulas have been proposed based on remodeling theory (Ahlqvist and Damsten, 1969; Singh and Gunberg, 1970; Iwamoto et al.,1978; Rother et al., 1978; Thompson, 1979; Hauser et al., 1980; Fangwu, 1983; Thompson and Galvin, 1983; Uytterschaut, 1985; Samson and Branigan, 1987; Drusini, 1987; Drunini and

Businaro, 1990; Narasaki, 1990; Ericksen, 1991; Stout and Paine, 1992; Kimura, 1992; Uytterschaut, 1985; Yoshino et al., 1994; Cool et al., 1995; Stout and Paine, 1996). The variety of published methods allows the researcher access to age estimation formulas for a number of different bones.

Use of Rib Microstructure for Age Estimation

This study used ribs for establishing histological age estimations. Ribs were chosen as they present several advantages over other skeletal elements. First, sections of rib are easily obtained at autopsy without additional manipulation of the decedent. Second, ribs are less susceptible to non-age related bone remodeling such as increased mechanical loads placed on the limbs (Frost, 1983; 1987a). Third, the entire cross section of a rib can be counted more easily then the larger surface areas of femurs and tibia. This is advantageous as sampling the entire surface allows for a more accurate estimate of OPD (Stout and Paine, 1992). This is important because of the interskeletal variability of osteon densities. "Two serial cross sections of rib with only 10 mm^2 cortical area each may exhibit several hundred percent difference in bone formation whereas two serial cross sections of femur with more than 300 mm^2 cortical area each will differ by less than 5%" (Wu et al., 1970). The importance of sampling the a large surface as possible surface is also supported by Lynnerup, Thomsen and Frohlich's study showing a high degree of intra and inter-observer variation when trying to establish histomoprhological criteria used to estimate age in a sample of femurs (Lynnerup, Thomsen & Frohlich, 1997).

Variables Affecting Bone Remodeling

Factors that influence remodeling can be divided into two categories. The first of these categories is heritable traits such as age, sex and ancestry. The second is environmental influences such as pathology, nutrition and drug use.

Age

The first heritable trait that has been shown to have an effect on bone remodeling is age. It is the infant that the highest rates of remodeling that can be expected to be found. This rate can be up to 30 mm^2 per year in early infancy (Jowsey, 1966; Martin et al., 1998). The rate slowly decreases throughout development and begins to decline as one reaches early adulthood. Once maturity is reached an adult remodeling rate of approximately 1 mm^2 per year is achieved (Martin et al., 1989). Remodeling will continue throughout adult life but at drastically lower rates than during the maturation phase. It is estimated that about five percent of compact bone is replaced by osteons each year after a person has reached their skeletal maturity (Martin and Burr, 1982). This remodeling will represent both baseline remodeling and response to microscopic damage (Frost, 1987b). The cause of accelerated remodeling in adolescents is likely the result of higher metabolic growth rates and hormones associated with the growth process (Martin and Burr, 1982). It is also possible that the higher rates of remodeling seen in younger persons may have something to do with the less mineralized nature of adolescent bones (Martin et al., 1989).

Sex

There are also sex based differences in the rate of remodeling. Females differ from males during growth and maturation with females on beginning their pubescent growth spurt earlier than males. Males typically trail females in their growth spurt by one to two years. Males also typically undergo a longer growth spurt which accounts for their larger size once they have completed the transition through puberty (Frost, 1997). Estrogen is required in both males and females for bone growth and maturation (Riggs, Khosla and Melton, 2002). In both females and males estrogen eventually causes the epiphysis to fuse thereby concluding bone growth (Riggs et al., 2002). However in males the addition of testosterone causes distinct differences in the resulting bone Testosterone causes males to gain up to twenty five percent greater bone mass by the time growth is completed (Riggs et al., 2002). There is also documentation suggesting that sex may play a role in the pace of remodeling rates. In Thompson's study of adult ribs he found that remodeling rates appear to occur at a higher rate in males when compared to females (Thompson, 1979). This was supported in another study that examined remodeling rates in femurs (Ericksen, 1991).

Remodeling rates change in women after the onset of menopause (Heaney, Reckler and Saville, 1978; Ousler, Kassem, Turner, Riggs and Spelsberg, 1996). Upon reaching menopause female remodeling rates increase rapidly when compared to males (Parfitt, 1979; Reckler, Lappe, Davis & Heaney, 2004). Some studies have concluded that sex based age equations are able to produce more precise estimations of age (Thompson, 1981; Ericksen, 1991).While some studies have shown no significant

differences between sexes when estimating age at death (Kerley, 1965; Stout and Paine, 1992; Stout and Paine, 1996).

Ancestry

A third variable that potentially affects bone remodeling is ancestry. Studies of differences in remodeling rates between populations have produced contradictory results (Han, Palnitkar, Rao, Nelson and Parfitt, 1997; Weinstein and Bell, 1988; Cho, Stout, Madsen and Streeter, 2002). Studies have indicated that African Americans have lower turnover rates than those of European descent (Han et al., 1997; Weinstein and Bell, 1988; Cho et al., 2002). These authors hypothesize that people of African descent may begin with more bone accumulated during growth. Since remodeling is a response to damage those with more massive bones may be less susceptible to mechanical stresses and thusly need to remodel at a much slower rate. However black Africans have been shown to have bone turnover rates more similar to whites then to African Americans (Schnitzler, 1993).

In another study Inuit were found to show greater turnover rates than U.S. Whites (Thompson and Gunness-Hey, 1981). Inuit have also been shown to exhibit greater bone turnover rates when compared to other Native American populations (Ericksen, 1973; Richman et al., 1979). As a result it has been suggested that population specific equations should be taken into account when doing age estimations (Thompson and Gunness-Hey, 1981). While ancestry has been shown to play a role in growth and development these studies demonstrate the lack of clear and discernable data as to what degree ancestry may affect bone remodeling.

Mechanical Stress

There is a correlation between increased mechanical stress and an increase in bone remodeling (Frost, 1983). As a bone undergoes the repeated stresses of daily life it slowly accumulates microscopic damage. Remodeling works to repair this microscopic damage caused by everyday wear and tear. If bones undergo excessive amounts of biostructural strain microdamages will accumulate at a faster rate hence increased remodeling. This increase in damage triggers an increase rate of repair through remodeling. Strangely the opposite of overuse also holds true. Significantly decreasing mechanical loads on bones also leads to increased remodeling (Mack and Lachance, 1966; Frost, 1987a). Individuals who undergo prolonged bed rest may develop changes in their skeletal makeup. Likewise, the decreased stress associated with space travel has been shown to have similar effects (Vico et al., 1988). In order to explain this paradoxical observation Frost developed the theory of a mechanostat which holds that there are mechanical strain thresholds, or minimum effective strains, that trigger specific responses" (Frost, 1987a). An important concept of this theory is that it separates modeling from remodeling when it comes to certain mechanical pressures. The mechanostat holds that once bone reaches a certain threshold for strain it enters a maintenance level of remodeling. When strains fall below this threshold remodeling is increased and bone loss occurs on the endosteal surface. This would explain why remodeling increases during times of disuse or severely decreased mechanical strain. Frost proposed that some remodeling continues to occur when bones are under normal stresses in response to microdamage. However, when stress exceeds beyond the maintenance level of remodeling the body responds by returning to the modeling process.

By switching to a modeling approach the bone can be altered in a manner which will improve its ability to handle the increased mechanical strains it has been put under (Frost, 1987a).

Dietary Influences

Proper diet and nutrition are known to be linked to bone quality. Nutrition plays an important role in the body's ability to respond to stress. Without the proper materials to support the metabolic processes used in the creation of bone the body will be unable to respond to damage in an effective manner. Lack of any number of nutrients can result in such disruptions. Too much dietary phosphorus can increase bone resorbtion by elevating parathyroid hormone (Kersetter, O'Brien and Insogna, 2004). Vitamin D deficient diets have been shown to be associated with bone loss (Fisher, Mitchell, Smiciklas-Wright, Mannino and Birch, 2004; Heaney, 2006, Ilich, et al., 2009). The appearance of rickets has long been recognized as a symptom of a Vitamin D deficient diet or lack of ultraviolet light exposure. In children suffering from rickets remodeling rates are increased as bone weakened by calcium deficiencies attempt to repair the damage they endure (White, 2000). Not only can deficiencies play a role in disrupting the remodeling process but poor diets can also play a role. For instance high protein diets can raise blood calcium levels in response to increasing pH (Kerstetter and Allen, 1990). Too often this increase in calcium comes at the sacrifice of skeletal material and a disruption of the remodeling process. It has been established that malnutrition is a frequent finding in a chronic methamphetamine abuser (Karch, 2002). Abusers frequently suffer from large drops in weight and may become anorexic. This chronic malnutrition undoubtedly affects the remodeling process (Schnitzler, 1993).

Sodium intake is also known to effect bone remodeling. High levels of sodium intake have been shown to increase the loss of calcium by altering the calcium balance within the body. If the imbalance persists long enough bone remodeling will decrease (Nordin, 1979).

Another study by Paine and Brenton (2006) studied the effect of dietary malnutrition on bone remodeling rates. Their study evaluated the Stout and Paine (1992) formula for age estimation against a sample South Africans (N=26) of who had known ages at death and were documented to be malnourished or suffer from pellagra (niacin deficiency). They found bone remodeling to be extremely curtailed producing histological age estimations on average 29.2 years below known actual ages (Paine and Brenton, 2006).

Pathology

Certain diseases and pathologies are known to influence bone remodeling (Frost, 1985). Studies have demonstrated that diabetics suffer not only from decreased bone formation but from a decrease in the rate of bone remodeling (Wu et al., 1970). Diabetes has been associated with a net loss of bone. Hongbing et al. (2003) reported that type I diabetes alters bone remodeling by reducing the formation of new bone, leading to osteopenia. This has been shown by a decrease in bone mineral density in humans and alterations in the formation of new bone in animal studies (Hongbing et al., 2003). The precise mechanism of how these disorders effect bone remodeling is not clearly understood.

Another interesting pathologic disorder that influences remodeling is that of osteogenesis imperfecta. Osteogenesis imperfecta is a genetic disorder that effects

connective tissues and increases the likelihood of bone fracture. Findings associated with OI are osteoporosis, gracile bones with thin cortexes and bowing of the extremities. Histological bone examination in OI has demonstrated decreased bone volume, cortical thickness and increased cortical and trabecular osteocytes (Dolinak, Matshes and Lew, 2005). This data is in agreement with increased activation frequencies associated with osteogenesis imperfecta were bones have a activation frequency at sometimes four times the rate of normal (Frost, 1963).

Hyperthyroidism is also known to play a role in bone remodeling. Thyroid hormone has a direct role in the resorption process in bones (Tortora and Grabowski, 1996). In persons suffering from hyperthyroidism bone resorption is increased due to an excess of thyroid hormone. Over long periods of time bone loss can result. This loss can eventually result in increased fracture risk in people suffering from hyperthyroidism (Tortora and Grabowski, 1996).

Psychological health can also play a role in bone health. Depression has been linked to bone loss through stimulation of the sympathetic nervous system in mice exposed to chronic mild stress (Yirmiya et al., 2006).

In response to these pathological conditions there has been some effort to create equations that correct for these conditions (Ericksen, 1991; Thompson, 1979). However pathological conditions can both accelerate or slow down remodeling rates (Robling and Stout, 2008).

Drugs

Certain chemical compounds are also known to play a role in the disruption of the remodeling process though by what exact mechanisms remains poorly understood. For

example large amounts of caffeine intake have been associated with decreasing bone density by inhibiting growth of trabecular bone (Hernandez-Avila et al., 1993). It was once suspected that caffeine led to increased excretion of calcium through urine. However this has proven not to be the case (Heaney, 2006).

The consumption of alcohol has also been shown to have negative effects on bone health (Toss, 1992). Chronic alcohol abuse is an important risk factor for risk of osteoporosis and fracture. While exact mechanisms remain to be clearly defined there is evidence to suggest that in the humans alcohol in large amounts is directly toxic to osteoblasts. This leads to a reduction in bone formation (Rico, 1990).

When alcohol is taken in moderation the opposite has been found to be true. Data predominantly for postmenopausal women does indicate a positive correlation between bone mass density and alcohol consumption (Felson et al., 1995). In another study women who drank moderate levels of alcohol were shown to have a higher bone mass then those that abstain (Turner and Sibonga, 2001).

Tobacco use has been shown to negatively impact bone quality (Fang, Frost, Iida-Klein and Hahn, 1991). Cigarette smoking was identified as a risk factor for osteoporosis more than 20 years ago. Subsequent studies have also demonstrated a direct relationship between tobacco use and decreased bone density (Sparrow, Beausoleil, Garvey, Rosner and Silbert, 1982; Toss, 1992; Fang et al., 1991; NIH, 2000). These studies are not without some controversy. Other factors may be at work that would explain differences between smokers and non smoker bone density (Toss, 1992). For example, smokers are often thinner than non-smokers. Smokers also tend drink more alcohol, may be less physically active, and often have nutritional deficiencies. (NIH, 2000).

Methamphetamine

Methamphetamine is an analog of amphetamine that was first synthesized in 1919 (Baselt, 2000). Methamphetamine has come under increasing attention as a drug of abuse over the past decade. However the history of methamphetamine abuse goes back much further. Methamphetamine was widely distributed during the Second World War to German troops as a means to provide an energy boost during battle. Methamphetamine was also widely prescribed as a miracle drug during the fifties for a host of ailments (Donaldson and Goodchild, 2006). Illicit methamphetamine production and use were first recognized as a social problem in the United States during the 1960's (Donaldson and Goodchild, 2006). Since then illicit use of methamphetamine has continued to increase despite substantial efforts in legislative and law enforcement activities. A 2004 survey found 50% of law enforcement agencies identified methamphetamine as the number one drug threat (Cohen and Sanyal, 2007). According to AmeraChem Incorporated (2006) the rise in methamphetamine abuse is due to several different factors. First, methamphetamine is relatively easy to produce with over one hundred and fifty different publications in chemical journals that are readily available to the public. The internet also provides a wealth of information to would be manufactures. The long lasting high provided by methamphetamine is another suspected reason for the drugs high incidences of abuse. The effects can last from four to twelve hours depending on the purity of the drug. Some highly concentrated forms of methamphetamine called "Ice" can create a high lasting up to sixteen hours. Ice as well as other forms of methamphetamine are relatively inexpensive when compared to other illicit drugs like cocaine. Lastly, it can

be easily created in small clandestine laboratories with all the ingredients readily available from commercial sources (AmerChem, 2006).

Methamphetamine has a stronger and more pronounced effect on the user than amphetamine and its effects last much longer (Spalding, 2006). Methamphetamine belongs to the family of psychostimulants called phenylethylamines (Baselt, 2000). Methamphetamines in is used in a clinical setting to treat a variety of ailments including attention deficient disorder, narcolepsy and obesity. Although methamphetamines do have clinical applications they are usually avoided or limited to short term use as tolerance to the drug builds rapidly (Beselt, 2000). The drug works by causing an increased release of nor-epinephrine release into the synaptic cleft which then overflows into the circulatory system resulting in sympathomimetic effects (Levine, 2003, Karch, 2002). This is accompanied by a release of both dopamine and serotonin (Levine, 2003). This cascade of neurotransmitters causes euphoric and pleasurable feelings. Methamphetamine can help to relieve fatigue, reduce need for sleep, increase energy and confidence levels and in general bring about psychological and physical exhilaration (AmerChem, 2006). People often abuse methamphetamines in order to achieve these side effects. The drug causes the user to feel an increased level of alertness and may engage in behaviors like repetitive cleaning and the assembling and disassembling of objects (DEA, 2008). It has also been shown that the drug may be abused for its weight loss properties (Cho and Melega, 2002). It is now estimated that 96% of the amphetamine found on the street is methamphetamine (AmerChem, 2006). Methamphetamine can be smoked, snorted, taken orally or injected (Spalding, 2006). A survey of

methamphetamine abusers found that the majority of abusers smoked the drug followed by injection as the second means of favored delivery (DEA, 2008).

Acute ingestion of methamphetamine produces a myriad of negative effects. Methamphetamine users are more likely to report impaired physical health then the general population (McKetin et al., 2008). Acute intoxication has been known to cause sudden death by stroke, seizure and cardiac dysrhythmia (Karch, Stephens and Ho, 1999; Beselt 2000). Other effects include hyperthermia, hypertension, rhabdomylosis, tachycardia and disseminated intravascular coagulation (Lora-Tamayo, Tena and Rodriguez, 1997; Gill et al., 2002). Overdoasage can also cause confusion, hallucinations, anxiety, convulsions, circulatory collapse and coma (Baselt, 2000). Chronic users can develop Magnon's syndrome in which they feel as if bugs are crawling underneath their skin (Spalding, 2006). This causes the abuser to habitually pick at their skin creating large sores that can become infected. Chronic abusers have also been shown to develop paranoid psychosis (Baselt and Cravey, 1995). Other psychological effects include suicidal behavior, delusions, hallucinations, aggressiveness, panic attacks and confusion (Cho and Melega, 2002). Abuser may suffer from seizures, anorexia, chest pains and possibly stroke (Baselt and Cravey, 1995). It has been shown that those that abuse methamphetamine are nine times more likely to be involved in a homicide (Stretesky, 2009). Methamphetamine users have been shown to have an increased risk of death from homicide, suicide and accident (Logan et al, 1998, Zweben et al., 2004).

The toxicity of methamphetamine is variable and there is no known acute minimum concentration that is known to be fatal in humans. Blood concentrations averaged .96 mg/L with a range of .09-18 in a series of 13 deaths attributed to

methamphetamine overdoseage (Logan et al., 1998). Depending on the duration of use suspected lethal concentrations vary widely from individual to individual (Mori, Suzuki and Ishiyama, 1992).

Addiction to methamphetamine is believed to be primarily psychological rather than physical in nature (Spalding, 2006). Due to the rapidly developing tolerance to the drug the user has to take increasingly high doses in order to achieve desired results (Cho and Melega, 2002). Methamphetamines initially produce physical pleasure, so users are easily seduced into the repeated use of the drug. Often users will continually take methamphetamines to avoid the "down" mood they get when the drug wears off (Spalding, 2006). Such behavior may cause the user to stay awake for days during which time personal hygiene may be adversely affected (Spalding, 2006). In addition to this lack of daily care methamphetamine may have detrimental effects on the bodies' ability to repair damage and cause damage and death of nervous tissues (Cadet et al., 2003). This can be seen in graphic photographs that have been taken to show the long-term effects of methamphetamine on a user's appearance (DEA, 2008). Such individuals seem to age rapidly and often appear much older after abusing methamphetamine for relatively short periods of time.

One of the most readily identifiable indicators of methamphetamine abuse is pronounced deterioration of the oral cavity. The phenomenon of "meth mouth" finds chronic methamphetamine abusers to have unusually high amount of damage to their teeth and gums (Richards and Brofeldt, 2000; Padilla and Ritter, 2008). Despite common belief, "meth mouth" is not due to methamphetamine being acidic or corrosive or by some sort of contamination from its manufacture as it is frequently seen in individuals

who ingest methamphetamine by injection (AmerChem, 2006). It is also not a product of impure processing as meth mouth has been observed in people who abuse pharmaceutical grade methamphetamines (Richards and Brofeldt, 2000). Instead the damage is likely the result of several different conditions known to exist in methamphetamine abusers. The first of these is xerostomia which in simple terms means dry mouth. Methamphetamine abuse results in decreased saliva production. This cause's accelerated damage as saliva provides a means of defense in preventing tooth decay (Klasser & Epstein, 2006). A second contributing factor to the deterioration seen in meth users is broken and cracked teeth. Methamphetamine abusers are known to frequently clinch their jaws tightly and grind their teeth (Donaldson and Goodchild, 2006). Such behaviors often lead to damaged enamel that puts the user at greater risk in developing caries. A third variable that may lead to the development of meth mouth is a general lack of oral hygiene. Methamphetamine abusers are known to consume large amounts of sugary caffeinated beverages and may abuse tobacco products during multiday binges (Klasser & Epstein, 2006). It has also been hypothesized that during the "crash phase" abusers may sleep for multiple days with their mouths open further increasing the damage from lack of saliva (Donaldson and Goodchild, 2006).

While it is difficult to know the exact number of methamphetamine abusers estimates have ranged from 600,000 to 1.4 million within the United States alone (AmerChem, 2006). Some law enforcement agencies estimate these numbers to be much higher. It is suspected that less than ten percent of illegal methamphetamine labs are ever discovered by law enforcement (DEA, 2008). These numbers suggest that methamphetamine abuse is an epidemic phenomenon. Multiyear studies have shown that

methamphetamine abuse has continued to increase over the last several decades (Cohen and Sanyal, 2007).

Methamphetamine and Bone Quality

Methamphetamine has been linked to lower bone densities (Katsuragawa, 1999; Kim et al., 2009). In a Japensese study conducted by Katsuragawa bone density was measured in prisoners who had a history of methamphetamine abuse. Sample sizes for both the control group and the test group contained at least fifty individuals. In the study the bone quality of the calcaneus bone was examined by ultrasound bone densitometer. Two measurements were taken one using the speed of sound and the other broadband ultrasound attenuation. These two measures are considered to be good indicators of the strength of bone. The study found that the bone densities of known methamphetamine abusers were statistically significantly lower than those of the control group (Katsuragawa, 1999).

Further evidence of decreased bone density in methamphetamine users was found in a recent Korean study (Kim et al., 2009). This study compared a group of hospitalized Korean male methamphetamine users (N= 46) to a sample of non-users (N=188). This study examined the lumbar spine using dual energy X-ray absorptiometry. The authors concluded that there was a considerable loss of bone in methamphetamine users and that abusers of the drug were more likely to suffer osteoporosis (Kim et al., 2009).

CHAPTER 3: MATERIALS

Methamphetamine User Sample

Transverse rib sections were obtained during autopsy at the Ada County Coroner's office in Boise Idaho by myself from individuals (N=18) who according to medical and or law enforcement histories were known chronic methamphetamine abusers. A waiver was obtained for each sample collected acknowledging each specimen may be used in scientific testing. A copy of this waiver can be found in appendix A. Each specimen was assigned a number 1-18. The age range for methamphetamine users was from 19-50 years of age with a mean age of 34.6 and a median age of 34.5 and a standard deviation of 9.7. A summary of age ragens can be seen in chart 3.1. Three individuals were shown to have tested positively for methamphetamine at the time of death. A summary of causes of death for the sample are displayed in chart 3.2.

Comparative Sample

A comparison sample (N=19) was assembled. Eight of these samples originated from the Boone/Calloway County Missouri Medical Examiner's office. The eight samples consisted of transverse rib sections that had been previously affixed to glass slides. These samples were designated in the study by their original case numbers. An additional nine samples were collected during autopsy at the Ada County Coroner's Office in Boise, Idaho. These samples were each assigned a letter A-K All individuals were selected based on no known history of methamphetamine abuse or metabolic

disease that may affect bone microstructure. The total sample (N=19) had an average age of 38.8 and a median age of 39 and a standard deviation of 10.94. Age ranges for each group are shown in Chart 3.1.

Chart 3.1 Age Ranges of Individuals

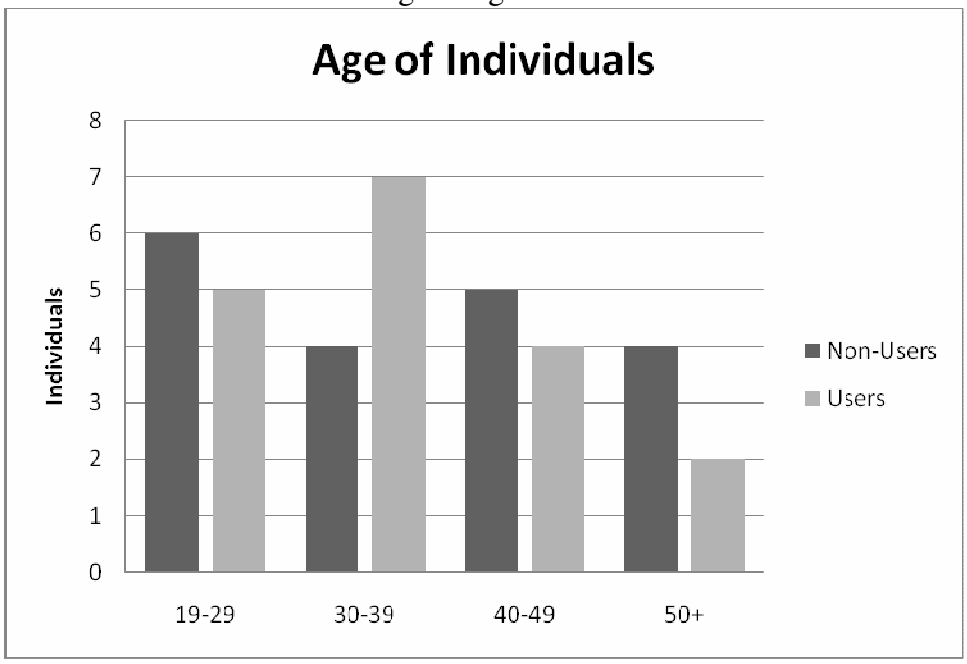
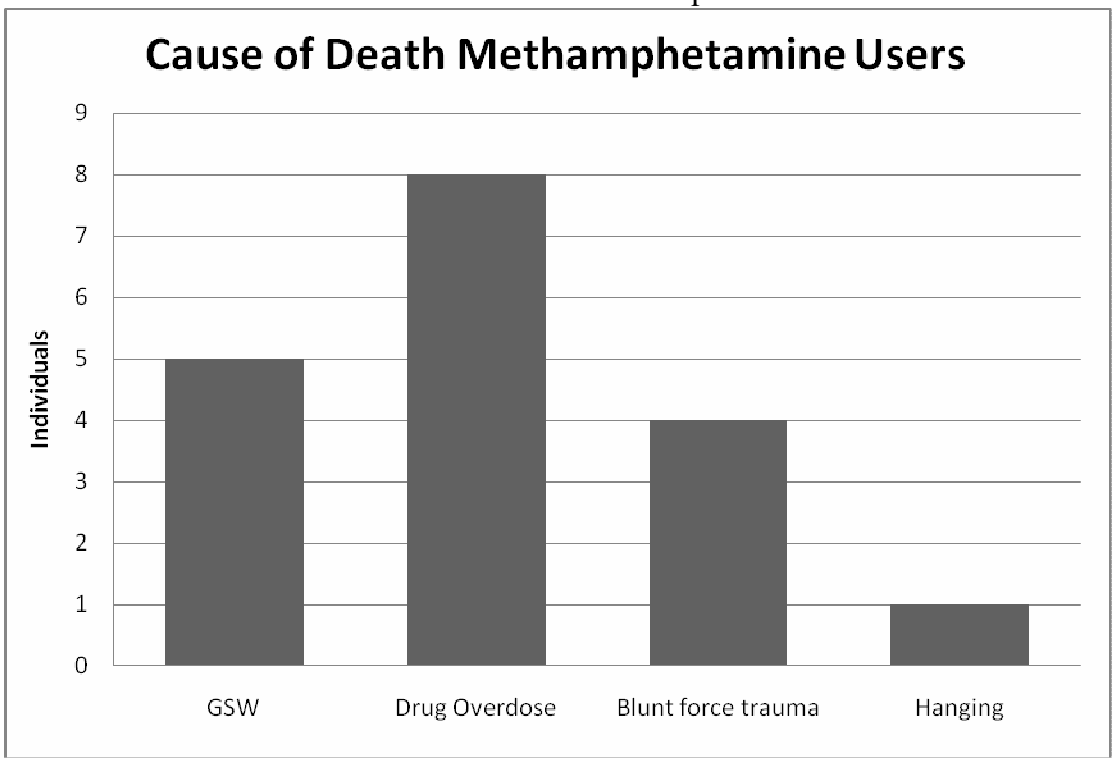


Chart 3.2 Causes of Death for Methamphetamine Users



CHAPTER 4: METHODS

Collection and Preparation of Materials

The samples included in the study that were obtained through the Boone/ Calloway County Medical Examiner's office and had previously been fixed onto microscopic slides using the method described below. All other samples were obtained at autopsy from the Ada County Coroner's office. These rib sections were soaked 24-36 hours in a Betty Crocker crock pot at 80 degrees Celsius to facilitate maceration of soft tissue. The sections were then gently cleaned to remove remaining periosteum.

Preparation of Slides

Preparation of slides followed standard histological procedures (Stout and Teitelbaum, 1976; Anderson, 1982; Stout and Paine, 1992; Parfitt, 1983; Stout and Lueck, 1995). Processed ribs were cut with a low speed metallurgical saw with a diamond embedded blade to a width of approximately 100 micrometers. Each of the sections was further hand ground with 400 grit silicon carbide paper to a thickness that facilitated microscopic viewing. The samples were then immersed in Clearite® to dregrease and facilitate better viewing of the slide. The sections were then affixed under cover slips to standard microscopic glass slides using Permamount®.

Microscopic Analysis

The samples were examined using the XLT Eclipse 80i Nikon microscope fitted with a Merz Counting reticule with polarizing light at 4x and 20x magnification. Mean

osteon size was recorded for each slide under 4x magnification. Cortical area and osteon population densities were calculated while using 20x magnifications. Mean osteon area, cortical area and OPD were calculated using the point count method. Data from these observations were recorded and entered into Microsoft excel spreadsheets in order to facilitate analysis.

Histomorphometry

Data was charted in order to identify possible outliers. Data was also charted in order to demonstrate relative differences when comparing mean osteon size, estimated vs. actual age, OPD to actual age, and cortical area to actual age. Regression analysis was undertaken in order to evaluate apparent trends for each of the three variables examined. The null hypothesis would predict that there is no statistically significant difference in each of the variables between methamphetamine users and nonusers. The null was tested based on observation of OPD, mean osteon size and cortical area. Differences between histologically estimated age and known ages were calculated and graphed.

Mean Osteon Area

Mean osteon size was the first variable measured. The mean osteon area or mean cross-sectional area (On.Ar) of an osteon is the average area of bone including Haversian canals contained within the cement lines of structurally intact osteons. In order to calculate mean osteon area fifty osteons are selected and each average area is calculated by using the point count method. Care was taken to ensure that osteons were sampled from all areas of the cortex. Only whole osteons with rounded Haversian canals were selected. The number of fields counted is multiplied by the area of magnification to

develop a possible area. To determine actual area the number of hits is divided by the possible hits which are then multiplied by the possible area. The calculation is as follows:

Actual Area= (# of actual hits/ # of possible hits) * possible area

A standard error difference of means test was used in order to determine whether observed differences between methamphetamine users and non-users were statistically significant. Using standard of error for difference of means carries with it a few important assumptions. First of all it assumes that both populations have a normal distribution. This is especially important in this case because of the small sample size being compared. It also assumes that both populations have equal variances (Caldwell, 2004). The value of the standard error of the difference of means was used to calculate a t-ratio. This t-ratio was obtained in order to determine whether differences in average osteon size between methamphetamine users and non users were statistically significant.

Cortical Area Calculations

Secondly, cortical areas were calculated for both methamphetamine users and non-users. Cortical area is a measure of percentage cortical bone that occupies a cross section of bone. Calculation of cortical area is accomplished using the point count method. To determine the cortical area (Ct. Ar) the endosteal area (Es. Ar.) is subtracted from the total subperiosteal area (Tt. Ar.) The Ct. Ar is then divided by the Tt. Ar and multiplied by 100 to determine the percent Ct. Ar. The formula is as follows:

Tt.Ar -Es.Ar = Ct.Ar

(Ct.Ar/ Tt. Ar) * 100 = % Ct.Ar.

Standard deviations as well as variances were also calculated. Since the data represented independent populations a standard error for the difference of means was

calculated. The value of the standard error of the difference of means was determined and used to calculate a t-ratio. This t-ratio was then obtained in order to determine whether the difference in cortical area in methamphetamine users was significantly different then nonusers.

OPD

OPD is the total number of intact and fragmentary osteons per mm^2. OPD was calculated following the methods described by Stout and Paine (1992). This formula required the calculation of osteon number, fragmentary osteon number and mean osteon area. This was accomplished through the point count method (Streeter, 2007). OPD was then calculated by dividing the number of whole and fragmentary osteons by the total area in mm^2. To determine OPD number of hits per field, number of intact osteons and number of fragmentary osteons is recorded for every other field over the entire cortex. Using this technique every other field of an entire cross section of bone is read. This results in a checkerboard type sampling pattern for reading slides. Osteons and fragmentary osteons on the grid margins with more than half of their structures included in the field were counted. OPD was calculated by summing the intact osteons with the fragmentary osteons and dividing this value by the total area. The formula for this equation is as follows:

OPD= (# of intact osteons + # of fragmentary osteons)/ total area (mm^2) area

Standard deviations as well as variances were also calculated for OPD. A standard error for the difference of means was calculated and used to calculate a t-ratio. This t-ratio was then used to determine whether the difference between OPD and known age between methamphetamine users versus nonusers was statistically significant.

Histological Age Estimation

In order to facilitate an accurate estimation as possible the Stout and Paine (1992) method of age estimation was employed. This method is the most widely used technique for deriving histological age at death estimations from human ribs. The Stout and Paine (1992) formula was created from a sample of primarily white individuals. This formula is based on a sample (N=40) of 32 white, four American blacks and four unknown. The age range for this sample was 13-62 with a mean age of 28.6. The sample was composed of 32 men, seven women and 1 individual of unknown sex. The average absolute difference between estimates and actual ages is 3.9 years.

The Stout and Paine (1992) formula for ribs is as follows:

$$Ln = 2.343 + .0508757 \text{ (rib OPD)}$$

The accuracy of this formula has been questioned when applied to other ethnic groups not included within the study (Ubelaker, 1977; Richman et al., 1979; Thompson and Gunness–Hey, 1981). For this reason only whites are included in this study in an effort to standardize the data.

CHAPTER 5: RESULTS

The results of this analysis are discussed in three sections. The first section shows results for osteon population densities with a separate section showing the impact on estimated versus actual age. Secondly, mean cortical area will be explored. Lastly will be a comparison of mean osteon size. An overview of basic statistics for each of the variables is found in Table 5.1

Table 5.1 Summary Statistics Methamphetamine Users vs. Non-Users

		Known Age (Years)	Histological Estimated Age (Years)	Difference (Years)	OPD (#/mm^2)	Cortical Area (%)	Mean Osteon Size (mm^2)
Meth Users	Mean	34.611	22.685	11.578	15.198	37.836	0.041
	Median	34.500	22.440	11.955	15.160	36.600	0.043
	Std Dev	9.720	3.942	7.785	3.489	7.367	0.005
	Variance			60.598	12.170	54.277	0.000
Non-Users	Mean	38.842	33.340	7.117	21.684	41.189	0.039
	Median	39.000	31.045	6.000	21.470	41.250	0.039
	Std Dev	10.946	10.738	6.379	6.644	6.485	0.003
	Variance			40.693	44.139	42.053	0.000

OPD

Osteon population densities (OPD) were obtained for 16 methamphetamine users and 18 non-users. Out of the original sample 9, 50 and J were not included in the analysis as errors in processing prevented an accurate osteon count from being obtained. Statistics for the sample of methamphetamine users and non-users are given in Table 5.2. Users

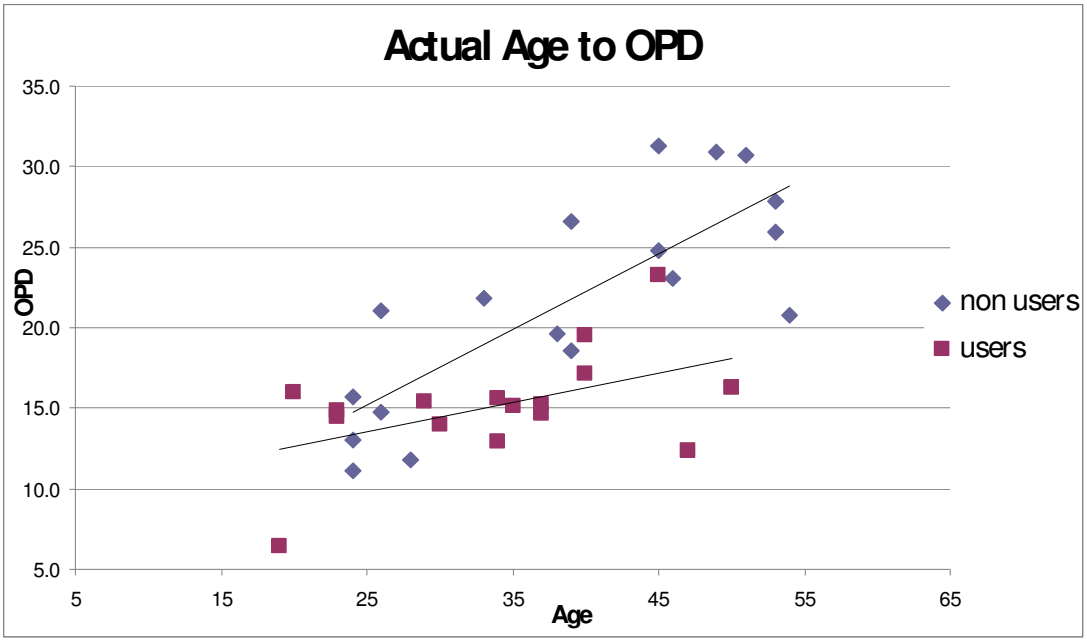
showed a mean OPD of 15.19 with a standard deviation of 3.48. Non-users were shown to have an OPD of 21.68 with a standard deviation of 6.64.

Table 5.2 Basic Statistics OPD Users vs. Non-Users

OPD (#/mm^2)	Users	Non-Users
Mean	15.198	21.684
Median	15.160	21.470
Std Dev	3.489	6.644
Variance	12.170	44.139

As shown in Graph 5.1 there is a positive correlation between the OPD and known ages for both methamphetamine users and non-users.

Graph 5.1 Osteon Population Densities (OPD) to Actual Age

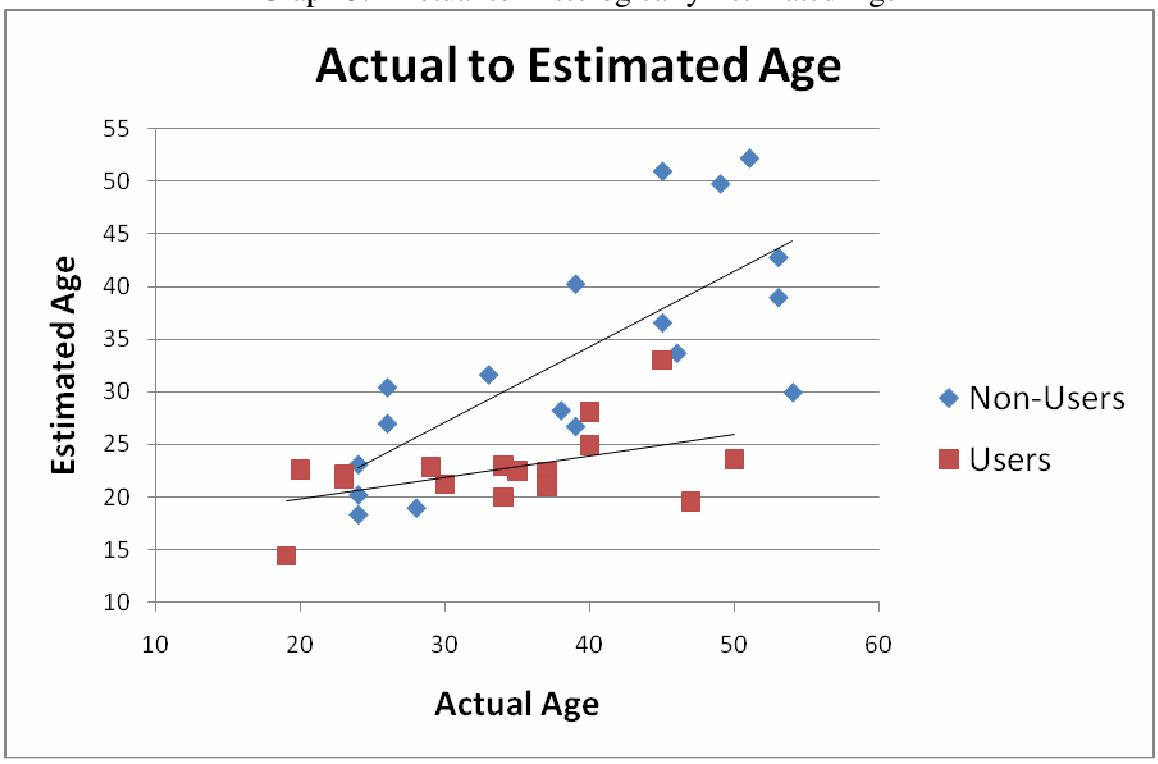


A standard error difference of means test was applied to the data. The value of the t-statistic was determined to be 3.50. This was greater than the critical value of 2.750

obtained for a p-value of .01. Elimination of the apparent outlier of case 5 (19yo, OPD of 6.41) results in a t-statistic of 3.33. This value is also greater than the critical value of 2.75 for a p-value of .01. In this case with or without the outlier one can say there is a statistically significant relationship at a 99% confidence interval.

Chart 5.2 shows actual to estimated ages. This graph shows a similar relationship to OPD to actual age. This is not surprising because estimated ages are based on osteon population density. This graph demonstrates that both non users and users tend to underestimated in age when using the Stout and Paine 1992 formula. However more accurate results are obtained for nonusers then users.

Graph 5.2 Actual to Histologically Estimated Age



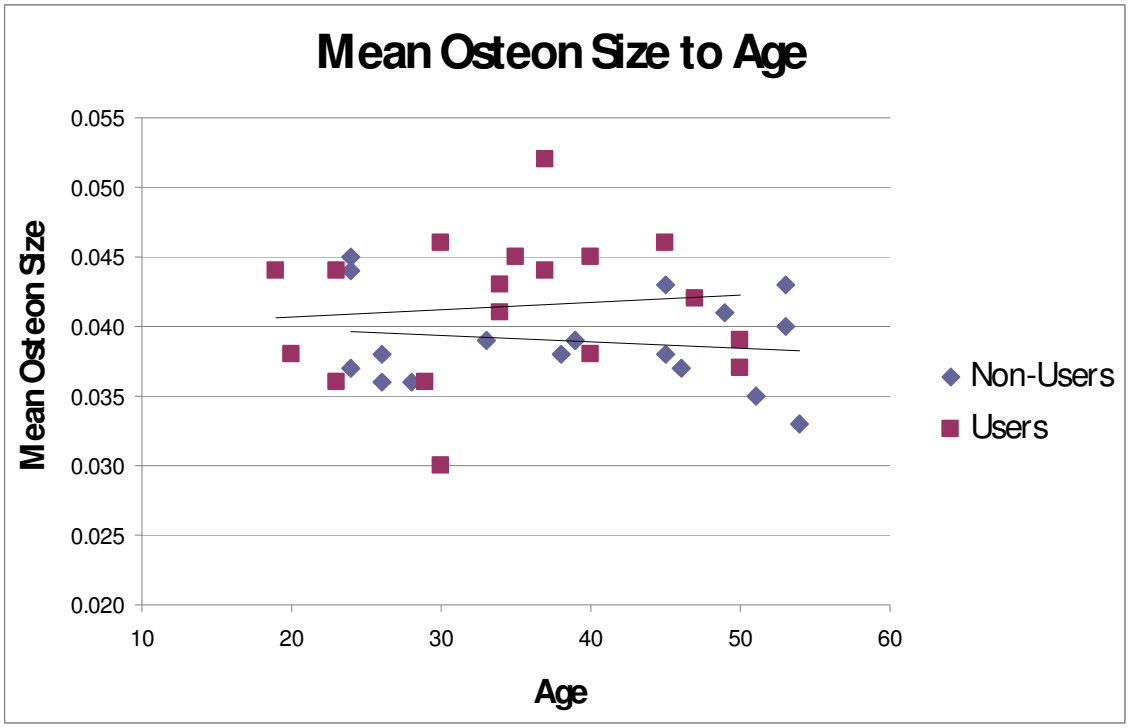
Mean Osteon Size

Mean osteon size was calculated for 18 methamphetamine users and 18 non-users. Sample J was not included for non users as accurate osteon size could not be determined. Basic statistics were calculated and presented in Table 5.3.Users had a average mean osteon size of .041 mm^2 with a standard deviation of .005 compared to non-users at .039 mm^2 and a standard deviation of .003 .

Table 5.3 Mean Osteon Size Users vs. Non-Users

Mean Osteon Size	Users (mm^2)	Non-Users (mm^2)
Mean	0.041	0.039
Median	0.043	0.039
Std Dev	0.005	0.003

Graph 5.3 Mean Osteon Size to Actual Age



The average size of an osteon in an adult human rib is .037 mm^2 (Wu et al., 1970). The methamphetamine user sample had an average osteon size of .041 mm^2 (Table 5.3). This is larger than the average for a non-user at .039 mm^2. As discussed earlier mean osteon size can be an indicator of overall metabolic rate (Abbott, Trinkaus and Burr, 1996). Smaller osteon size is associated with less vigorous osteoclast activity. In this study methamphetamine users have larger osteons then those of non-users.

A standard error a difference of means test was calculated in order to determine whether the results were significant. The t-statistic calculated confirms that there is a statistically significant difference between the two populations at the .10 level of significance. The calculated value is 1.70 which exceeds the critical value of 1.697 at a .10 level of significance. However when calculated at the .05 level of significance a value of 2.042 level is obtained which does not meet the test for statistical significance. In the case of mean osteon size one can assume there is a relationship at a 90% confidence interval but not at a 95% confidence interval.

Mean Cortical Area

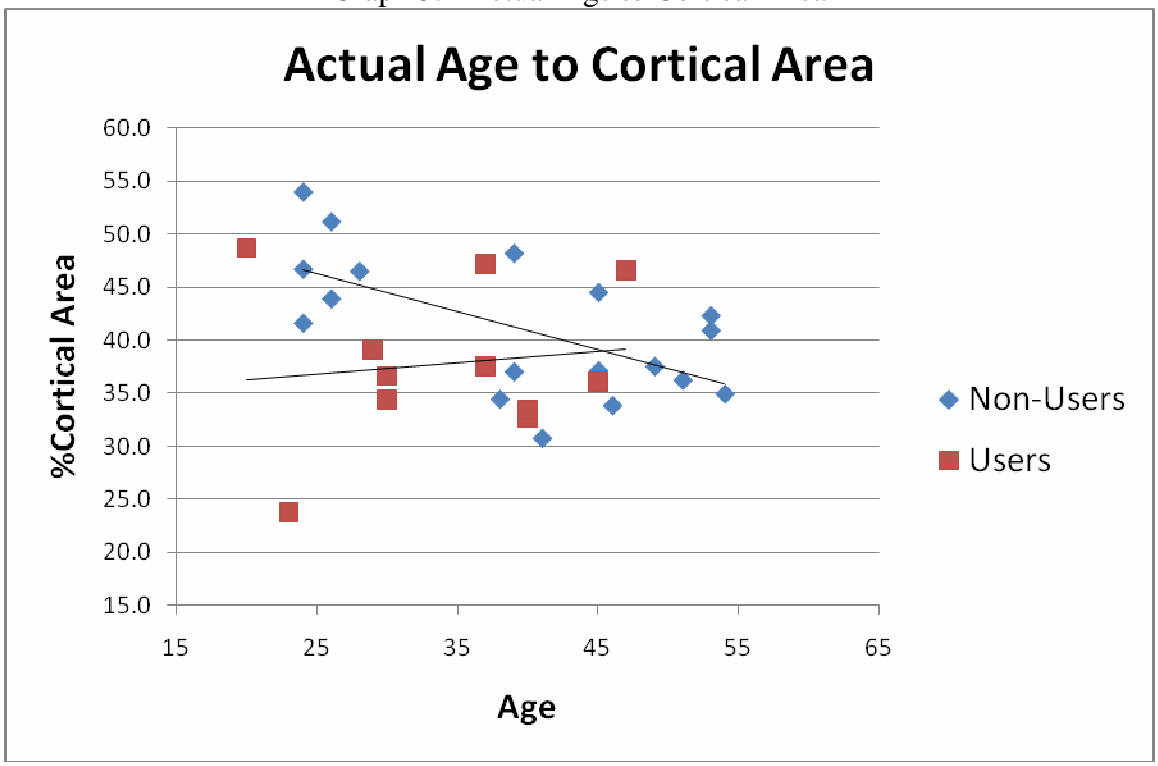
Cortical areas were calculated for 11 methamphetamine users and 18 non-users. Cortical areas were not obtained for specimens 2, 4, 5, 8, 11, 12, and F due to breaks in cortical bone during processing. Basic statistics were preformed and are represented in table 5.3. The mean cortical area for users was 37.84% with a standard deviation of 7.37 cortical area compared to non-users 41.19% cortical area with a standard deviation of 6.48.

Table 5.4 Basic Statistics Cortical Area Users vs. Non-Users

% Cortical Area	Users	Non-Users
Mean	37.84	41.19
Median	36.60	41.25
Std Dev	7.37	6.48
Variance	54.28	42.05

The data was plotted on graph 5.4. Non-users appear to follow a trend of decreasing cortical area over time. Methamphetamine users on the other hand appear to slightly increase cortical area as they age. A standard error difference of means test was calculated to determine whether the differences between the two populations was statistically significant. A result of .566 was calculated which is not statistically significant even at a .20 level of significance or at an 80% confidence interval.

Graph 5.4 Actual Age to Cortical Area



CHAPTER 6: DISCUSSION

OPD

Osteon population densities are lower in methamphetamine users then in the general population at statistically significant relationship with a 99% confidence interval. Reduced rates of remodeling are also associated with both environmental and heritable forms of stress (Wu et al., 1970; Cook, Molto and Anderson, 1988 Paine and Brenton, 2006; Fisher et al., 2004; Heaney, 2006; Ilich et al., 2009; Toss, 1992; Jowsey, 1966; Martin et al., 1998; Heaney et al., 1978; Ousler et al., 1996). More importantly it has been demonstrated that certain types of drugs have an effect on bone quality (Hernandez-Avila et al., 1993; Toss, 1992; Rico, 1990). Methamphetamine abuse is also known to cause other conditions that may contribute to reduced remodeling rates such as poor nutrition and poly-substance abuse (Baselt, 2000; Cho et al., 2002; Spalding, 2006; Donaldson and Goodchild, 2006; Richards & Brofeldt, 2000, Padilla and Ritter, 2008). Because of this the exact mechanism that causes this reduction in remodeling is not clear. It is unknown if this reduction is the result of the methamphetamine itself or the result of a lifestyle associated with drug abuse.

Implications for Histological Age Estimations

The low OPD associated with methamphetamine users has serious implications for those attempting to do age at death analyses based on histological characteristics. In this study estimated ages varied on average 11.57 years in meth users compared to 7.2

years in nonusers (Table 5.3). In both samples there was a tendency to underestimate age. This is similar to other studies that have shown that pathological conditions can affect bone remodeling and therefore age estimations (Wu et al., 1970; Cook et al., 1988 Paine and Brenton, 2006). For example Paine and Brenton's study showed niacin insufficiency could lead to underestimating the age of an individual on average of 29.2 years (Paine and Brenton, 2006). This study demonstrates that individuals who abuse methamphetamine are likely to be under aged when evaluated on histological criteria of the rib.

Mean Osteon Size

Mean osteon size can be an indicator of overall metabolic rate (Abbott et al., 1996). Smaller osteon size is associated with less vigorous osteoclast activity (Abbott et al., 1996). In this case it appears that methamphetamine users have larger osteons then those of non-users at a .10 level of significance or 90% confidence interval. However when calculated at the .05 level of significance or 95% confidence interval level the differences do not meet the test for statistical significance. In the case of mean osteon size one can assume there is a relationship at a 90% confidence interval but not at a 95% confidence interval. An increase in sample size may help to resolve this issue. A known side effect of methamphetamines is an increase in metabolism while under the influence of the drug (Volkow et al., 2001; Levine, 2003; Karch, 2002). However methamphetamine use is also associated with prolonged periods of inactivity during the crash phase. For this reason the exact mechanism in which methamphetamine abuse would result in larger osteon size in unknown and a potential topic of future investigation.

Cortical Area

Greatest cortical area is known to coincide with peak bone mass (Takahashi and Frost, 1966). The amount of cortical area follows a pattern of increasing from birth through the attainment of peak bone mass during the second decade of life (Sedlin et al., 1963). Once peak bone mass is reached cortical area slowly decreases throughout life due to increasing expansion of the endosteum (Sedlin et al., 1963; Takahashi and Frost, 1966). As demonstrated in graph 5.3 this trend towards a reduction in cortical area is recognized in the non-user population. However, when methamphetamine user data is graphed in graph 5.3 it appears that the opposite trend is occurring with cortical area slightly increasing over time. However, when the two populations are tested against one another they are not shown to be statistically different even at a .20 or 80% confidence interval. The slight increase in cortical size in methamphetamine users may be a byproduct of small sample size of methamphetamine users (N=11). Further investigation with a larger sample size may be able to help more clearly define this issue.

CHAPTER 7: CONCLUSIONS

Mean osteon size, cortical area and osteon population densities were calculated for a group of methamphetamine users. When compared to a group of non-users it was found that osteon population densities were significantly different in those that abused the drug. Osteon size also possibly varies in methamphetamine users. In this study cortical area does not seem to vary in methamphetamine users.

Osteon population densities were found to be significantly less in methamphetamine users ($p < .01$). OPD that are less than predicted for age indicate some disruption of the remodeling process (Wu et al., 1970; Cook et al., 1988; Paine and Brenton, 2006; Fisher et al., 2004; Heaney, 2006). This has important implications when histological age estimations developed from OPD are conducted on meth users. As demonstrated estimated ages for those that abuse the drug are less accurate then non users. When calculated these age estimations have a tendency to underage individuals by an average of 11.6 years.

Mean osteon size was found to be significantly larger in methamphetamine users at a 90% confidence interval ($p< .10$). However this difference was not significant at a 95% confidence interval ($p > .05$). Larger osteons are associated with increased metabolic activity (Abbott et al., 1996). Methamphetamine intoxication is associated with increased metabolism (Volkow et al., 2001). Larger than average osteons in the meth users might first suggest increased metabolic activity and thus higher rates of bone remodeling.

However the fact that bone is remodeling at a rate slower in methamphetamine users would tend to suggest the opposite. The underlying mechanism behind this phenomenon is unknown and warrants further analysis. Increasing sample size may also work to make the study more statistically robust.

Size of cortical area did not vary significantly between populations ($p > .20$). Cortical area did tend to decline over time in the non-user sample. In the methamphetamine user sample cortical area slightly increased with age. However, cortical areas tended to be smaller to begin within the methamphetamine users. This particular variable suffered from a small sample size ($N=11$) due to damage to the cortex during processing on several of the samples.

When determining whether methamphetamine plays a role in bone microstructure one needs to consider the array of other conditions that a methamphetamine abuser may have had exposure too. Methamphetamine abuse is known to be positively correlated with unsafe sex practices (Spalding, 2006). One should consider that the abuser may be suffering from a variety of different illnesses such as HIV, syphilis, hepatitis and other sexually transmitted diseases. This is especially true for methamphetamine abusers who may use injection as their preferred method of abuse. Sharing needles can cause the abuser to be exposed to any number of communicable diseases that all may play their own role in negative bone health. In addition substance abusers frequently do not remain exclusive to one particular type of drug (Bourne, 1974; Clemens, Mcgregor, Hunt and Cornish, 2007). As demonstrated certain drugs are known to affect bone remodeling (Toss, 1992; Hernandez-Avila et al., 1993). More detailed medical histories concerning

substance abuse and the length and severity of methamphetamine abuse would certainly be beneficial.

The exact mechanism in which methamphetamine affects bone quality is unknown. This is true of other variables that have also been shown to be correlated with negative bone health. These include heritable traits such as sex, age and ancestry as well as environmental factors such as poor nutrition, drug use and disease. However, the one thing all of these examples have in common is they represent some type of stress. Stress is known to affect physiological processes. As remodeling is but a physiological process it is not surprising that even non-specific stressors in the environment could potentially manifest themselves in the skeleton. Even if these stressors do not appear to play a direct role in bone metabolism they could affect some process that eventually cascades to the level whereby bone is affected.

This concept holds especially true for those who habitually abuse narcotic drugs like methamphetamine. While the exact mechanisms on how chronic methamphetamine abuse effects bone metabolism may not be clearly understood the stress placed on the abusers body through factors such as lack of hygiene, exposure to infectious diseases, malnutrition and others can all be recorded within skeletal material.

One recommended way in which future research can be bolstered would be to examine sections from other skeletal elements (Robling and Stout, 2008). The biggest hurdle to such an approach is obtaining samples. Some work has already been completed looking as the potential of more non-invasive approaches to studying bone histology. Stout demonstrated that it is possible to develop three dimensional images for use in bone histology (Stout et al., 1999). The use of micro-CT to re-create cortical bone topography

has been shown to be a possible avenue for evaluating bone microstructure and estimating age (Cooper, 2005).

While advances in technology will eventually solve some of the problems with obtaining samples continued study is still possible. Increasing sample size will undoubtedly improve an understanding of a methamphetamines relationship to bone histomorphometry. This study has shown that there is a correlation between methamphetamine use and alterations to the microstructure of ribs. It is possible that further study will shed light into the exact mechanisms behind this phenomenon. In the meantime caution should be taken when histological age analysis is undertaken on suspected meth users and unknown individuals because of the apparent influence that a methamphetamine lifestyle has on OPD.

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APPENDIX

Waiver Form

WAIVER FORM

Ada County Coroner's Office
Policy Statement and Request to Release Body and Organs

The Ada County Coroner's Office will release the body to the next of kin or person authorized by law to receive the remains. Further, the Ada County Coroner's Office has the authority under Idaho Code Title 19, Chapter 43 to investigate deaths and perform autopsies. An autopsy is a scientific inquiry by a medical professional that involves an external examination of the body and a surgical dissection so that internal tissues and organs can be removed, examined, and subjected to scientific testing. In most cases, remains of organs are returned prior to the release of body for burial. Bodily fluids and tissue samples kept for microscopic examination and/or testing are not returned. On rare occasions, the Ada County Coroner's Office must retain one or more whole organs (usually the brain or the heart) for extended periods of time to complete examination and testing. Because these tests can take several weeks to complete, the body is often released for the purposes of burial or cremation prior to the return of the organs.

This material may be kept for evidence in determining the cause and manner of death for any related criminal case. In addition, upon a written request from an attorney, the Ada County Coroner's office may keep this material for evidence in a related civil case. Upon completion of the autopsy and any related criminal and/or civil case, the next of kin will be permitted to obtain this material from the Ada County Coroner's Office within six (6) months or it will be disposed of in accordance with policy set by the Ada County Coroner. The Ada County Coroner's Office may be contacted at 208-287-5556 to inquire if any organs were retained.

Request to Release Body and Organs

I hereby request that the Ada County Coroner's Office release the body of the above-named deceased to: _____

Funeral Home (or other organization)

Further, I understand that I may contact the Ada County Coroner's Office and instruct them of the my preferred method of disposition of any organs that may have been kept for further study by the Ada County Coroner's Office (please note that depending on the method of disposition, there may be fees involved). I understand that I must obtain this material from the Ada County Coroner's Office within (6) months or it will be disposed of in accordance with policy set by the Ada County Coroner (at no cost).

I hereby certify that I am the next of kin to the person named above, or other person authorized by law to receive the remains, and I have full authority to act on his/her behalf. I have read and understood the above information and hereby request the Ada County Coroner's Office to follow the instructions contained herein.

Printed Name: _____ Date: _____

Signature: _____ Relationship to the Deceased: _____

Witness: _____