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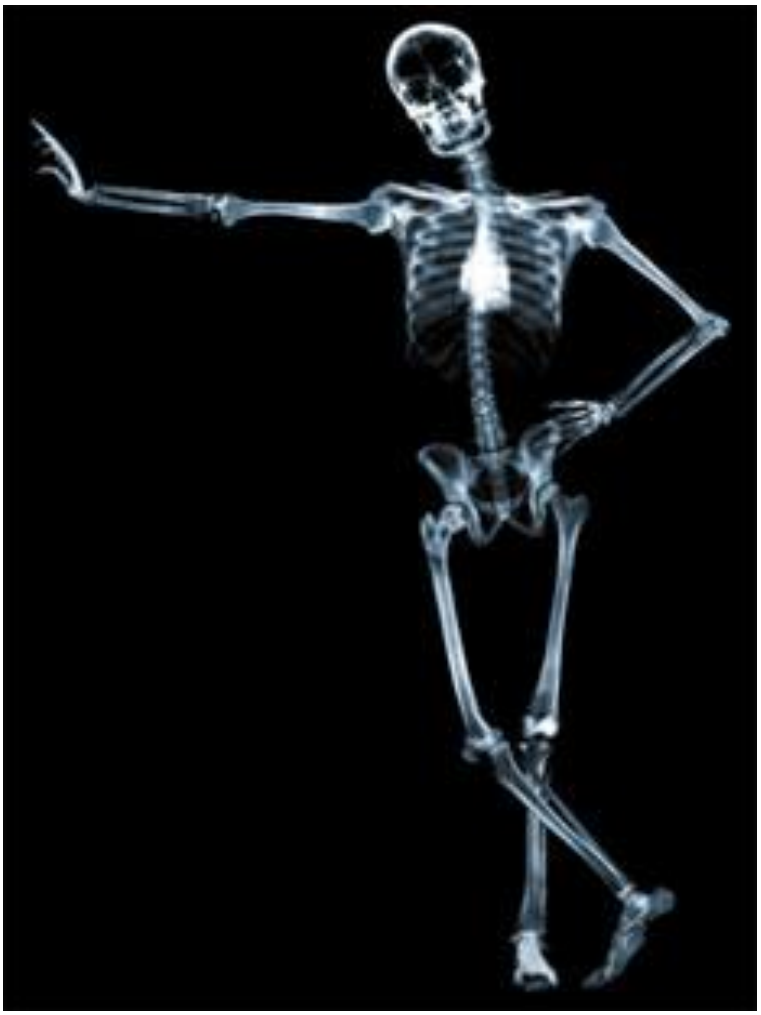
An exploration of Quantitative Computed Tomography (QCT) and Dual-Energy X-ray Absorptiometry (DEXA) assessments of age-related bone loss in a 19th Century Dutch Sample.



Rachel J. Olsthoorn

MSc Thesis

Bone Loss in Osteoarchaeology:

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Fishie, Here's looking at you kid!

List of Abbreviation

BMC – bone mineral concentration
BMD – bone mineral density
$_{a}$BMD – areal bone mineral density
$_{v}$BMD – volumatic bone mineral density
BMU – bone multicellular unit
BV/TV – total bone volume
Conn – connectivity; number of trabeculae
ConnD – connectivity density
DEXA – dual-energy x-ray absorptiometry
EYA – early young adult
LYA – late young adult
MA – middle adult
OA – old adult
QCT – quantitative computed tomography
ROI – region of interest
TbSp – trabecular spacing
TbTh – trabecular thickness

1 Introduction

With age comes change. When we are young, we grow strong and tall. However as we get older our hair starts to gray, our skin wrinkles and new aches and pains develop. The physical external changes occur in subtle increments and as we notice them we often try to 'improve' our exterior appearance through materialist means, as has been done for centuries. Yet, what happens to our bones? Although we cannot see them, they are in constant motion through phases of remodeling that, like our exterior, deteriorates. As we age our bone mineral density (BMD) decreases because osteoclasts, bone resorption cells, resorb bone faster and more efficiently than the osteoblasts, bone formation cells, can lay down new bone (Parfitt 2003). Osteoarchaeologists see individuals as they really are, not the external manipulation of beauty, but the internal structural components that make up our skeleton. Through the understanding of how an individual's bones changes with age, we can provide useful and detailed information about past activity patterns, nutrition, environmental stressors, and overall health of a population.

1.1 Defining Bone Loss

The clinical term osteopenia, in its most simplistic definition, is bone loss. Everyone experiences bone loss as we age, adapt to external stresses, and deal with the consequences of other diseases and disorders. However, osteopenia is more complex than just bone loss. Osteopenia is a metabolic disorder that can be defined as a condition in which a decrease in bone mineral density (BMD) is greater than the normal population variation but less than the risk of fracture. Osteoporosis is the advanced form of osteopenia and is defined as "a disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to enhanced bone frailty and a consequent increase in fracture risk. (Engelke *et al.* 2008, 130)" The difference between normal and osteoporotic bone can be seen in figure 1. BMD is a measurement of the mineral content in grams (BMC) within the region of interest (ROI) in centimeters (Carey and Delaney 2010; Hassager and Christiansen 1995) and is expressed as:

Area BMD $\quad BMD(g/cm^2) = \dfrac{BMC\ (g)}{Area\ (cm)}$

Volume BMD $\quad BMD(g/cm^3) = \dfrac{BMC\ (g)}{Area\ (cm) * Area\ thickness\ (cm)}$

The differentiation between areal BMD as $_aBMD$ and volumatic BMD as $_vBMD$ will be made throughout this thesis to help clarify the difference between each type of data reading as suggested by Engle *et al.* (2008).

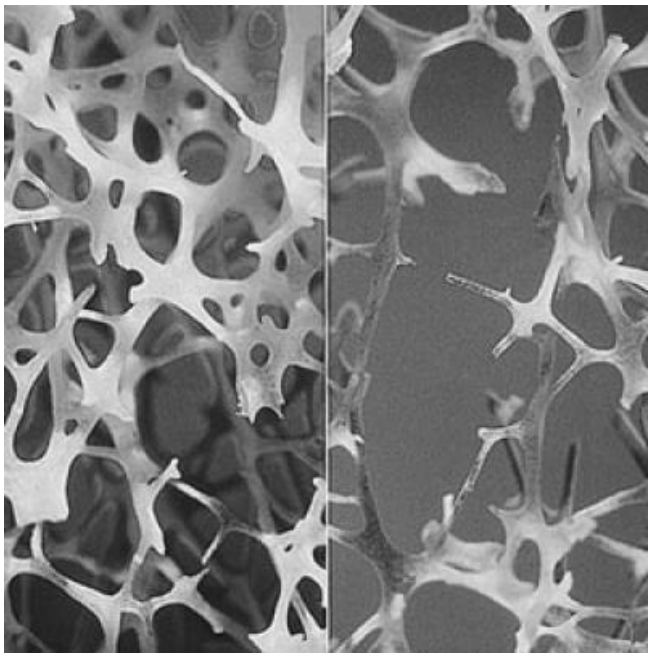

Figure 1: Normal trabecular architecture on the right, osteoporotic on the left. Image from the Osteoporosis Foundation.

BMD is monitored in modern populations to determine rates of osteopenia and osteoporosis as part of diagnosis and treatment. Specialized software (Heaney 2005) that has been available since the 1980's (Adams 2008), i.e. DEXA (dual-energy x-ray absorptiometry) provides accurate assessment rates of bone loss for individuals and groups. The World Health Organization (WHO) defines osteopenia as a BMD t-store between -1.5 and -2.5 standard deviations (SD) with anything greater than -1.5 SD being normal and less than -2.5 SD considered osteoporotic (WHO Geneva 2003, 40). It should be noted that t-scores are based

on age-related decrease and standard deviation from DEXA only (Englke *et al.* 2008) and an individual's stature, body mass and ethnicity need to be imputed to obtain reliable data (see 2.2.2.2 non-mechanical influences for more information about how these aspects affect bone loss). Other diagnostic methods such as QCT and different elements than those that are designated for this type of assessment (lumbar vertebrae and the proximal femur) can not use the WHO t-score definition (Adams 2008; Prevrhal *et al.* 2008). The t-scores rate for osteoporosis, less than -2.5 SD, was set to identify the arbitrary level of 30% of the post-menopausal female population as having osteoporosis (Adams 2008).

Osteopenia can be further subdivided into primary and secondary. Primary osteopenia consists of age-related changes to the body such at hormonal changes (menopause), loading stresses (excessive and in-excessive) experienced during growth, and nutritional factors. Secondary osteopenia is caused by compilations associated with other conditions such as immobilization due to injury or pathology (Brickley and Ives 2008). Primary and secondary subdivisions for osteoporosis are the same as osteopenia (Brickley 2002). Age related bone loss causes an increase in marrow cavity size and a decrease in cortical and trabecular thickness thus weakening bone and increasing fracture risk (Frost 2003).

The term osteopenia has been heavily used in archaeological literature with little formal review. It is often misused in defining bone loss with no further explanation as to primary or secondary relevance. A prime example of this can be seen in the work from Signoli *et al.* (2002) where the term osteopenia was stated as part of the pathology examination for individuals found in a mass grave who were affected by the plague during the 18th century in Provence, France. The term was never used again throughout the article. The authors noted there was "marked thinning of the corticals and refraction of the trabecular bone (*ibid*, 837)" in individuals over age 50 however no formal correlation of bone thinning and the term osteopenia was made. It can be inferred that the two are linked however the correlation implied by the authors is vague and unclear. Lewis (2010) determined the presence of osteopenia based on visual examination of cortical thinning as part of a thalassaemia diagnosis for individuals from a Romano-British archaeological

assemblage from Poundbury Camp in Dorset, England. However, the term osteopenia was used as general bone loss with no division between osteopenia, and osteoporosis as well as how taphonomy affected or caused cortical thinning before examination. It was clearly stated that the assemblage was poorly preserved.

Due to the complexity of the term osteopenia that is based on modern populations as an actual disorder diagnosis, this thesis will use the term "bone loss" and not osteopenia unless it is indicated by other researchers. This is contradictory to Brickley and Ives (2008) who state "any detectable bone loss that appears greater than normal in bioarchaeological studies should be considered to be osteopenia.(*ibid*, 152)" The question then becomes, what are normal bone loss rates for past populations? This thesis's sample is not adequate to answer this question and thus the term osteopenia will not be used. Additionally, for archaeological material there are too many factors that play into bone loss, either ante-mortem or post-mortem, that can not be clearly accounted for due to the complex dynamics of the body itself and the burial environment. In archaeological contexts, osteoporosis can often be diagnosed (macroscopically) by the presence of Colles fractures (distal radial), femoral neck fractures, and thoracic and/or lumbar vertebral compression, wedge, and/or concave fractures; in conjunction with bone thinning (Brickley and Ives 2008; Roberts and Manchester 2007). However, fractures are not always present and thus an individual that could have osteoporosis might be missed. Further investigation into aspects of bone geometry, architecture and physiology are needed for the determination of osteoporosis. Therefore, like the term osteopenia, osteoporosis will only be used when it is used by other researchers or there is a clear fracture present that is associated with the diagnosis of osteoporosis (Brickley and Ives 2008).

1.2 Literary review of bone loss detection methods

Macroscopic, microscopic (including scanning methods), and histological methods can be used to examine skeletal remains for bone loss. Macroscopic assessment of bone loss is a visual examination of fracture or sectioned edges, microscopic examination used scanning techniques such as micro-CT, QCT, and

DEXA and/or microscope assessment of the bone matrix, and histological methods examine thin/thick bone sections either through imaging techniques or under a microscope. These methods are used to examine bone quality aspects of bone loss to determine fragility (fig. 2). Microscopic and histological procedures are the best methods to determine bone loss. Agarwal (2008) reviews the pros and cons of the main techniques that can be used for the examination of archaeological material. Her research shows that each method has its own complications resulting from differences between skeletal elements, measurement location, machine calibration, external factors such as taphonomy, and technician error. Imaging methods are non-destructive; however, certain types of microscopic and histological examinations can be minimally destructive. Macroscopic assessment is destructive only if bones are purposefully sectioned/broken, a practice that is rarely encountered and highly discouraged.

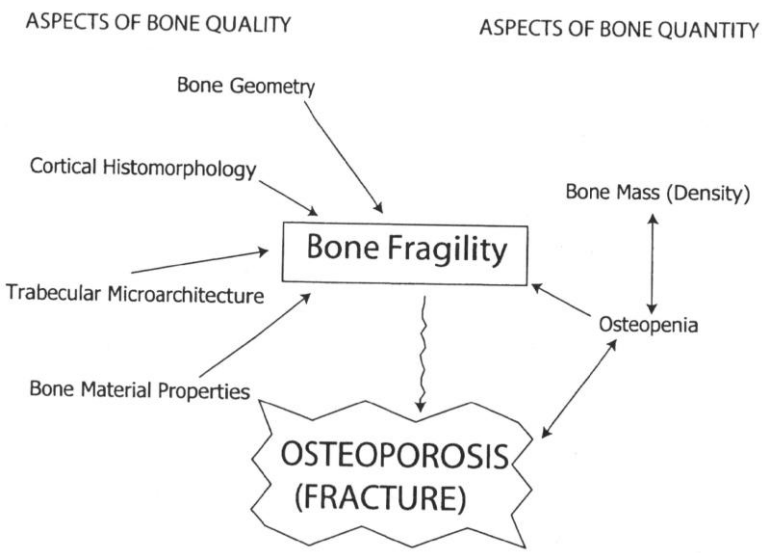

Figure 2: Interaction of bone quality aspects and bone quantity aspects in bone fragility (Agarwal 2008, 391).

When examining bone loss macroscopic examination of fracture sites is unreliable at best. The inability to clearly examine cortical and trabecular bone in healed or partially healed fractures makes this method ineffective. Damage caused during burial, excavation and/or storage can erode an ante-mortem fracture site and present a blurred picture of the fracture site and the internal structure. Micro

and gross fractures due to an original fracture can cause increased bone loss though secondary damage and breakage. In short, even if you have a young healthy individual and an old individual both presenting clean, clear fractures only a general determination can be suggested that one has less bone. Not osteopenia, but actual less bone at the fracture location due to any number of factors (taphonomy, disease, damage, etc). Caution must be taken when interpreting bone loss at ante-mortem and post-mortem fracture sites.

The exceptions are the three osteoporostic fractures: Colles fractures, lower vertebral compression fractures, and/or fractures of the femoral neck (Brickley and Ives 2008). Brickley (2002) examined investigation methods, clinical information, archaeological bone analysis, and historical record methods, of osteoporotic fractures from 18th and 19th century individuals from London, England. Her research indicated that multiple research sources provide a different picture of past fractures than only pathological determination. Archaeological evidence of femoral neck fractures are rarely present while current clinical information and historical records indicate that their presence was known as well as the problems associated with them such as massive blood loss, shock, and in many cases (up to 40%) death within six months of the initial trauma. Colles' fractures are more prevalent within the archaeological record. Written records (past and present) provide a well developed understanding of treatment as well as with the past suggest that distal radial fractures have low mortality and morbidity rates. However past populations would have been left with wrist deformation and minimal functionality. Of all three osteoporotic fractures discussed, compression fractures of the vertebra are most commonly observed within archaeological assemblages. Current research indicates that past populations would not be dramatically impaired by vertebral fractures and little historical information is associated with them.

Drusini *et al*. (2000) examined cross-sectional femoral cuts of sixty-six adults from the Veneto Region in Northeast Italy to examine osteoporosis within the Longobards people residing in this region in 760 AD. They concluded that gradual femoral osteopenia was present for both males and females, with females

more pronounced until age fifty, even though femoral shaft structural architecture was maintained. The destruction of archaeological elements is not done lightly. It is preferred to explore non-destructive methods such as CT scans which could have been used to obtain the same cross-sectional information.

Dual-energy x-ray absorptiometry (DEXA or DXA) measures an element's mineral context and its density to determine BMD. For current populations, DEXA, originally implemented in the 1980's (Adams 2008) is the main method to determine the presence of osteopenia and/or osteoporosis in an individual. The system has a precision rate of 1 – 2.5% depending on which element is scanned with the main reading taken at the lumbar spine and proximal hip (Damilakis *et al.* 2007; Symmons 2004). However, data obtained from archaeological material is questioned because of the alteration of mineral content as a result of diagenesis (Mays *et al.* 1998). Yet, even with diagenesis, Mays *et al.* (1998) determined that significant age related bone loss, can be seen through femoral BMD in the Wharram Percy medieval population, England. Their assessment indicated that lifestyle may not be as influential as age. Continued investigation of the Wharram Percy assemblage will be discussed in detail later in this chapte,r as multiple methods have been employed to understand the site. Gültekin *et al.* (2008) obtained BMD from two hundred and fifty five well preserved femura of individuals aged 15 to 45+ years from eleven archaeological sites across Anatonia dating from approximately 5500 BCE (Chakolithic age) to the 19th century. Their research indicated that proximal femoral mean BMD was lower in females than males for all ages with both males and females showing a decrease in BMD as age increased. This study examined individuals from a large time period, spread over a large region that is culturally and genetically diverse. It must be questioned if this data is valid to compare hunter gatherer, agricultural and more modern sedentary groups over time. The study of Gültekin *et al.* (2008) can indicate a change in BMD over time however this data remains vague because further variables cannot be specifically accounted for with any consistency such as diet, physical activity, cultural and/or environmental stresses.

Quantitative computed tomography (QCT) creates a 3-dimentional volumetric representation of a scanned element. This provides the researcher with multiple options for BMD determination in that cortical and trabecular tissue can be separated so that rabecular structure can be seen (Damilakis 2007). Gonzales-Reimers *et al.* (2007) examined QCT tibial bone mineral density from 78 prehistoric individuals from Gran Canarioa and El Hierror. Additionally, histomorphometric analysis was conducted on the tibial sample to assess trabecular bone mass. Their data indicated that QCT was not a promising scanning method to evaluate osteopenia with past populations because it only provides a rough estimate of trabecular bone mass. However, previous DEXA scans by the authors on the tibial sample indicated that DEXA and QCT correlations are statistically significant. Their assessment is interesting because they suggest that QCT is not a good method to evaluate bone loss when compared to histomorphology yet it is significantly correlated to DEXA. This conclusion is contradicting but at this time, no other archaeological literature assessing bone loss with QCT was found.

Metacarpal radiogrammetry utilizes x-rays of the second metacarpal to determine the percentage of cortical bone to its overall width. The use of the second metacarpal does not indicate rates of fractures related to frailty because it is a non-load bearing bone. This is important to note because load bearing bones are affected more heavily by loading stresses that can weaken them. When compared to a "healthy individual" cortical thickness provides a general assessment of skeletal health (Symmons 2004). The original technique was developed for clinical use in the 1960's (Barnett and Nordin 1960) and it has been utilized for archaeological material for the past few decades. Early utilization of this method by Mays (1996) determined that medieval Wharram Percy post-menopausal women showed significant cortical bone loss. Comparisons were made with a modern sample as well as historical literature which indicated that there is a close link between the stress factors that women face from the past and the present. Rewekant (2001) also used this method on two medieval Polish populations (n=219 Cedynia, burial ground in the northwest and n=145 from a

rural cemetery in Slaboszewo) to show the connection between environmental stress and bone loss. Individuals who experienced increased stress during childhood had less bone mass in adulthood. Ives and Brickley (2004, 2005) have coined this method by developing a procedural guide for its use in bone loss assessment of past populations. Their research indicates that the cortical bone measurements taken from the second metacarpal provide a good measurement of bone loss for non-load bearing elements. Additionally, this element "is characterized by relatively small morphological variability (Rewekant 2001, 437)."

Cross-sectional geometry is used to measure the morphology as well as cortical thickness of a bone through x-ray, CT scans and/or cross-sectional cuts. Bridges (1989) early work examined the cross-sectional geometry of femoral cortical bone through CT scans to determine the morphological changes caused when indigenous peoples shifted subsistence strategies from hunting and gathering to agriculture. Her research of Archaic hunter-gathers and Mississippian agricultural groups from northwest Alabama, USA, indicated that while bone strength increased, female cortical morphology was redistributed with activity changes. This suggests that while bone morphology changed, the level of bone loss and the rate of osteopenia would not be altered.

Trabecular architecture examination looks at microstructural changes in trabecular bone by using CT scans, x-rays and/or thin sections. An examination of trabecular architecture, using micro-CT, was conducted on the capitate and navicular of twenty individuals from the Anglo-Saxon cemetery, Raunds Furnells, Northamptionshire, England, by Macho *et al.* (2005). Their data indicated trabecular thinning could be linked to lower BMD, more prevalent in females than males, thus correlated to the presence of osteopenia and osteoporosis. Agarwal *et al.* (2004) examined trabecular architecture by radiographing lumbar vertebral thin sections from a medieval British assemblage to determine bone quality related to age, sex, and physical activity. Their data clearly displayed a link between the role of physical activity and thinning of trabecular bone.

Histomorphometry is a destructive method that uses thin or thick slices to

examine the micromorpholgy of bone. Roberts and Wakely (1992) examined vertebral cortical histormorphology of medieval Romano-British and English skeletal material (*n*=4; two females, two males) to view the changes of bone loss and further correlate the changes to historical literature. While destructive, their analysis showed that trabecular thinning and microfracture calluses were present prior to external signs of osteoporosis. Gonzales-Reimers *et al.* (1998) examined right tibial histormorphomety at the midpoint of the diaphysis and determined that the values for osteopenia correlated with cortical index measurements for individuals (*n*=133 prehistoric; *n*=41 prehispanic) from Gran Canaria Island. They concluded that a large number of prehistoric individuals had osteopenia. However, it has been suggested that this may be a biased assessment because of the use of dry bone which can potentially provide lower histomorphometric results. As technology progresses, high resolution CT scan can hopefully provide us with a non-destructive tool to look at histomorphology.

Current research regarding bone loss in archaeological assemblages is contradictory. This small sample of literature illustrates this, with each author using different elements and techniques to answer their research questions. Each method provides different data sets that are often not comparable to each other. The use of modern medical equipment is another problem because it is hard to calibrate for soft tissue and dry bone as well as individual machine calibration. Additionally, elemental selection also affects the type of data produced because each element has its own unique mix of cortical and trabecular bone.

Over the past two decades S. Mays, S. Agarwal, R. Ives, and M. Brickley have been the forerunners in the study of age-related bone loss (osteopenia and osteoporosis) in archaeological assemblages and have focused their efforts on the medieval Wharram Percy site in England, a pre-industrial population characterized by a rural medieval lifestyle (Agarwal and Grynpas 2009). Parish records were found providing ages of birth and death as well as familial ties. They have utilized different methodologies to determine bone loss through metacarpal radiogrammetry (Mays 1996; Ives and Berkley, 2004; 2005), discussed above, and DEXA (Agarwal and Grynpas 2009; McEwan *et al.* 2005; Mays *et al.* 1998).

Agarwal and Grynpas (2004) examined 58 (27 male, 31 female) fourth lumbar vertebra for sex and age BMD changes (excluding those with vertebral fractures) using thick slices scanned with DEXA. Their study indicated vertebral trabecular bone loss in young adult males and females with males exhibiting bone loss later in life than females. Male trabecular BMD decreased steadily across age groups, while females exhibited increased bone loss earlier in life with no change in BMD from middle to old age. Additionally female BMD was not significantly less than males for all ages, and postmenopausal BMD was not severely lower as that would be normally expected. These conclusions are supported by Agarwal *et al.* (2004) trabecular architectural assessment for this population. Mays *et al.* (1998) scanned 144 proximal femura with DEXA to obtain BMD and took radiographs of the femoral diaphyses to study the correlation between trabecular rich and poor sites. Femoral data indicated significant age-related bone loss similar to modern populations even though both experienced different lifestyles; no fractures were observed in the Wharram Percy assemblage. The authors suggest that osteoporotic severity may not be affected by lifestyle as much as generally believed.

As QCT has recently become more accessible for archaeological use there is still little utilization of this method at this time. Its non-destructive, high resolution 3D images present a promising turning point in the study of archaeological BMD and bone geometry (Brickley and Agarwal 2003). The availability of this technology in conjunction with the well preserved skeletal assemblage from Middenbeemster, Netherlands, provides a unique opportunity to explore QCT's advantages and disadvantages when compared to the gold standard of DEXA. Additionally, historical records and other current research on Middenbeemster will give us a better understanding of bone loss for this population.

1.3 Research Questions

To better understand age-related bone loss in a 19th century agricultural community, the Middenbeemster sample evaluated and the following questions will be assessed.

Can age related bone loss be measured in an agricultural archaeological assemblage by the determination of BMD of load bearing (femur) and non-load bearing (humerus) skeletal elements through the use of DEXA and QCT? If BMD can be determined, is areal aBMD(DEXA) or volumetric vBMD(QCT) a better indicator for age related bone loss in archaeological material? What can QCT assessment of trabecular bone volume, thickness/spacing and connectivity tell us and how is it comparable to DEXA BMD?

If a good indicator for bone loss can be determined by these methods (DEXA and/or QCT), then further subquestions can be addressed.

- What are the rates of bone loss for the Middenbeemster population? Is there a marked shift between normal, osteopenia and osteoporosis for males and females and at what ages do these shifts occur? In all, what can the data tell us about age related bone loss for this population? What are the BMD differences between load bearing and non-load bearing elements from the past?
- Are there different rates of bone loss between males and females, and is that pattern similar to what is documented in modern populations? That is, Are modern standards comparable to small agricultural groups from the 18^{th} and 19^{th} century?

The purpose of this thesis is to grasp a better understanding of QCT and DEXA's diagnostic role in age-related bone loss in past populations. The Middenbeemster assemblage is unique in that it consists of a well preserved skeletal material from all ages with accompanying historical records. This thesis will not only bring clarity to QCT and DEXA's roll in past population analysis but also help paint part of the bigger picture of bone loss within agricultural populations.

2 Bone Histology and Age-related Bone Loss

At some point within life, you will be affected by age related bone loss. However, although this concept is universal, not everyone will develop osteopenia at the same time. The study of age-related bone loss, osteoporosis and its precursor osteopenia, has increased over the past few decades because of its increasing diagnosis in modern populations. A complex web of interacting factors, which are only partly understood, play into age related bone loss. This chapter provides a review of basic bone histology and physiology. An emphasis is placed on the non-mechanical influences that are currently known to affect physiology and can be determined when working on archaeological material.

2.1 Bone Histology

Bone forms the structural framework for our bodies. It serves as mechanical levers for muscles and as marrow and mineral storage containers (Karaski 2008) for compounds such as phosphorus, sodium, and calcium (Aiello and Dean 1990). The skeleton's main mineral component is calcium at 99% (Brickley and Ives 2008). Bone is divided into two main types: cortical and trabecular. Cortical bone is dense and solid making up the external portions of bone. Trabecular bone consists of a light honeycomb structure that makes up the interior of many of the skeletal elements except for the medullary cavities of long bone shafts which are relatively hollow. Cortical and trabecular tissue are identical in composition but differ in their structure, i.e. their level of porosity (White and Folkens 2000). Bone tissue is made up of approximately 25 % organic collagen and approximately 70 % inorganic hydroxyapatite ($_3Ca_3(PO_4)_2.(OH)_2$) (Burton 2008; Waldron 2009). The interaction of collagen and hydroxyapatite provide bone with its strength, rigidity and hardness (White and Folkens 2000). An adult skeleton consists of approximately 20 percent trabecular bone and 80 percent cortical bone, however each skeletal element varies considerably (Agarwal 2008; Karaski 2008).

2.2 Bone Physiology

Throughout our lives our bones constantly change at the microscopic level through modeling during childhood, and remodeling cycles which take over in adulthood, to repair and maintain bone (Waldron 2009). In both remodeling and modeling, resorption of bone by osteoclasts and formation of new bone by osteoblasts takes place (Waldron 2009). As presented by Parfitt (2003), the function of modeling is the redistribution of equal quality bone at different locations through a continuous sequence of formation and resorption during growth to produce net bone gain. Therefore, increasing bone strength but not decreasing it (Frost 2001). Peak bone mass (bone mass achieved prior to the onset of remodeling at the climax of an adolescents growth spurt) is achieved between 15 and 35 years of age (Brickley and Ives 2008). After maturity, remodeling takes over. Old bone is replaced because new bone is needed due to structural fatigue of peripheral elements with a low turnover rate or metabolic over-mineralization of axial elements (Prevrhal *et al*. 2008) with a high turnover rate at a specific location (Brickley and Ives 2008). A sequence of activation, resorption, reversal, and formation commences in a cyclical pattern resulting in net bone loss (Waldron 2009). The remodeling cycle either stabilizes or decreases strength but can not increase it (Frost 2001). "Bone strains caused by muscle force bone indirectly but strongly influence modeling and remodeling effects on a bone's strength (Frost 2001, 238)."

Bone Multicellular Units (BMU) are the temporary structures that carry out remodeling throughout the skeleton. Whether in conservation mode (equal formation and resorption) or disuse mode (increased resorption and decreased formation), only disuse mode affects the bone directly next to marrow (Frost 2001). Unlike modeling where there is a constant sequence of formation and resorption, remodeling requires activation and all osteoclasts and osteoblasts in a mature individual belong to the BMU. The BMU is created when remodeling at a specific location is needed. Precursor cells are created for localized osteoclasts, osteoblasts, and the BMU's supporting connective tissue. As the BMU tunnels into cortical bone, the forward cone consists of osteoclasts followed by

developing osteoblasts which move from the center outward to form a three-dimensional necklace of workers to fill in the resorption areas and deposit osteoid (unmineralized bone) throughout the process (Brickley and Ives 2008). Mineralization starts 10 to 15 days after osteoid deposition (Brickley and Ives 2008). A cycle of activation, resorption and formation averages four months producing approximately 0.05 mm^3 of bone (Frost 1998; 2003) annually renewing 25 percent of trabecular bone and two to three percent of cortical bone (Aiello and Dean, 1990). A healthy individual's BMD decreases less than 1% per year, menopausal women up to 3% per year (Kangetal 2005). Complete skeletal turnover occurs approximately every ten years (Waldron 2009).

Remodeling of the trabecular bone takes place on top of the interconnecting web of bone rather than through tunneling (Parfitt 2003). This process is similar to cortical remodeling except that no osteoid is deposited because trabecular bone is fed through diffusion, absorption of nutrients from surrounding tissue (Brickley and Ives 2008). In general, through the remodeling process, trabecular resorption cavities are under-filled while the resorption cavities on the outer surface of the cortical bone are over-filled causing, with age, an increase in bone diameter and trabecular cavity space, resulting in trabecular thinning (Aiello and Dean 1990). Current QCT research indicates males and females can experience trabecular bone loss in early adulthood (Agarwal and Grynpas 2009). Additionally, trabecular bone loss is increased in non-load bearing bones (Roberts and Wakely 1992). The physiological bone loss changes to cortical and trabecular bone are summarize in Table 1.

Table 1: Observable physiological bone changes present with bone loss. Indented and italics changes for cortical bone, osteoporosis, are factors that are affects of cortical bone loss. (after Brickley and Ives 2008, 259 and 182)

Tissue type	Osteopenia	Osteoporosis
Cortical	↑ Number of resorption pits	↑ Porosity
	↑ Resorption pit depts	↑ Bone loss
	↑ Resorption pit fusion	↑ Resorption pit fusion
	Incomplete osteon filling	↑ Thinning
	↑ Number of micofractures	↑ *Trabecular structure*
	↑ Fatigue damage	↑ *Medullary cavity*
		↑ Damage
Trabecular	↑ Resorption, thinning	↑ Thinning
	↑ Number of microfractures	↑ Microfractures
	↑ Spacing	↑ Spacing
	↓ Connectivity	↓ Connectivity
		Trabecular thickening
		↓ Remodeling through surface removal
		↑ Damage

2.2.1 Reversible and irreversible bone loss

The remodeling cycle will continue throughout an individual's life, but with age comes change. Age related bone loss is characterized as disordered remodeling with a reversible and an irreversible component. Parfitt (2003) indicates that in reversible loss, increased remodeling causes a relocation of calcium stores from one area to another. This process is prevalent during early growth and later during pregnancy and lactation when calcium is temporally removed from the bones to help facilitate these needs. Thus, an increase in reversible loss will cause a decrease in bone mineral density and mean bone age that will go back to normal when the remodeling sequence returns back to normal.

Irreversible loss occurs when resorption and formation rates are not equal (fig 3). This is caused either by an increase in osteoclast activity causing a deeper resorption pit than normal with normal osteoblast filling or normal osteoclast resorption and a decrease in osteoblast activity causing incomplete bone filling of the resorption pit (Parfitt 2003). In both cases, the imbalance creates a small concavity at the resorption site during the remodeling cycle that cannot be reversed. This process is heavily influenced by muscle strength rather than body

weight (Frost 2003) and "presumably the disuse-mode remodeling causes all adult-acquired osteopenias on earth (Frost 2001, 239)."

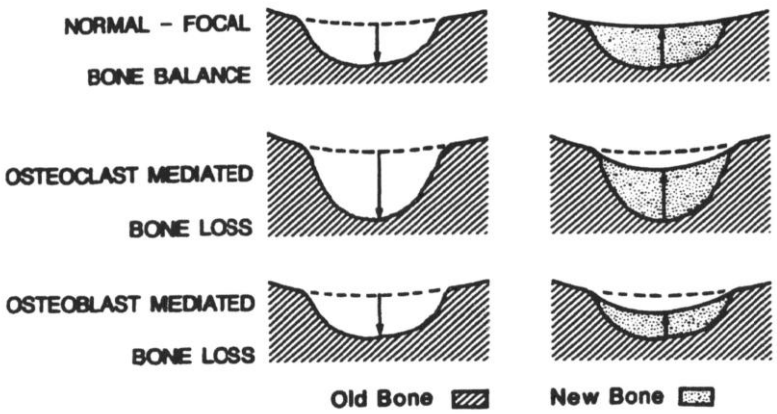

Figure 3: Normal, osteoclast and oateoblast remodeling imbalance. (Parfitt 2003, 12)

2.2.2 Physiology concepts: past and present

Wolff's law states that "every change in the form and function of the bone or of their function alone is followed by certain definite changes in their internal architecture, and equally defined by their external conformation, in architecture with mathematical laws (Wolff 1892 cited by Frost 1998, 600)." While this law is known within the osteoarchaeological field, its message is incomplete because it does not take into account non-mechanical factors that affect bone architecture such as genetics, nutrition, and hormones (Frost 1998). Increased age causes structural changes that are both mechanical, such as physical stresses from activity, and non-mechanical, such as hormones. Therefore the "Utah Paradigm" and the Mechanostat concepts, explained below, must be taken into account when studying age related bone loss.

The Mechanostat concept indicates "excepting infection, trauma and neoplasms, in all amphibians, birds, mammals, and reptiles of any size, age, and sex, the strengths of their bones, joints, ligaments, tendons, and fascia adapt to their voluntary mechanical usage in ways that keep them from breaking or hurting for life (Frost 1998, 602)." In other words, bones adapt to the stresses we put them through to decrease fracture risk. This statement, while elegantly put, is not so

simple. Non-mechanical factors affect, either positively and/or negatively, architectural adaptation. Figure 4 illustrates the negative feedback loop of these factors based on the mechanostat concept. Additionally, these changes can increase the risk of many disorders that can cause an increase risk of fracture, such as osteopenia and osteoporosis. For example, muscle strength is often overlooked yet it is an important factor of skeletal development, disease and overall individual health (Frost 1998). Loading stimuli on load bearing elements cause progressive resorption of trabecular bone while non-loading bearing elements will exhibit an increase in bone loss and ultimately increased fracture risk when falling (Brickley and Ives 2008). It should also be noted that the mechanostat concept does not apply to some skeletal elements; cranial bones what experience little or no loading (Frost 2003). The Utah Paradigm is a constantly evolving concept where the interaction of all tissues is being connected though multi-disciplinary research, striving for a better understanding of skeletal pathology (Frost 2001). This paradigm in essence is presenting mechanical and non-mechanical factors as an interacting spider web. What may seem simple to understand, such as age related bone loss, in fact is an extremely complex process.

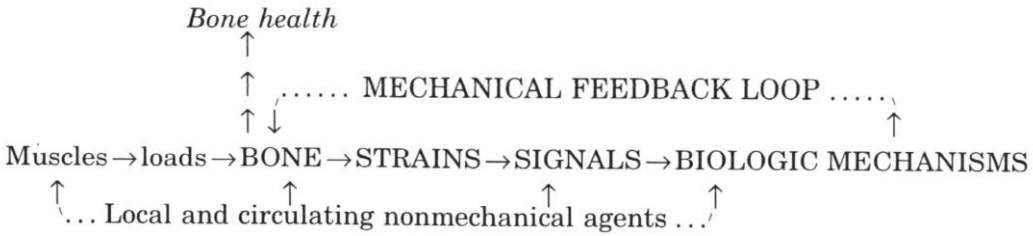

Figure 4: Negative feedback loop, based on the mechanostat concept, illustrating the non-mechanical and mechanical correlation to bone health (Frost 2001, 238).

2.2.2.1 Mechanical influence

Originally believed to be controlled by effector cells (osteoblasts and osteoclasts), mechanical influences are now thought to be governed by "tissue-level nephron equivalents (Frost 2003, 20)". That is, mechanical factors are governed by structural and functional units that are vital for bone health because no other cell can perform such function. Bone "nephron equivalents" use effector

cells within modeling drifts and remodeling BMU cycles (Frost 2003). The mechanical aspect in figure 4 above is in direct correlation to net bone loss or bone gain through modeling and/or remodeling cycles caused by physical strains exerted on bone. As bone structure changes and bone mass decreases, bone strength is significantly reduced, increasing fracture risk (Brickley and Ives 2008).

2.2.2.2 Non-mechanical influences

Non-mechanical influences that effect skeletal physiology are those that are considered natural (table 2: primary influences) such as age, hormones, genetics, and nutrition. A more complete list of factors can be found in Frost 1998, 2003. However, archaeological material is influenced by postmortem factors as well as ante-mortem ones. Therefore, Table 2 is divided into primary, secondary, and tertiary influences for a better understanding of how these factors pertain to past populations. Primary influences are those that are general factors that can affect skeletal physiology and include aspects that are not always discernible within past individuals but make up the basis of physiological bone change that is supported by modern population studies, such as menopause. Secondary influences are factors that cause bone loss as a side effect. This category includes conditions such as immobilization, rickets and various metabolic diseases. Those that are discussed below are present within the population sample used for this thesis as determined through paleopathological analysis. Both primary and secondary influences affect an individual before death. Tertiary influences are strictly postmortem. These factors are considered when working with archaeological material (taphonomy, excavation, and storage damage) that affect bone preservation.

In most cases, factors that are divided into primary and secondary are combined into a general category of ante-mortem influences. However, when dealing with archaeological material this division is important because while there are many influences, not all of them can be detected within an assemblage and therefore are assumed as general knowledge or not presented. For the purposes of this thesis, the break-down of factors into primary, secondary, and tertiary categories will help provide a better understanding of what influences bone loss. It

is not one influence that works alone to cause change, rather it is the interaction of influences that cause bone loss. A short description of each influence that can be determined through archaeological analysis is presented below.

Table 2: Examples of primary, secondary and tertiary influences of bone loss. A compilation of factors from Agarwal 2008; Brickley and Ives 2008; Frost 1998, 2000, 2003; White and Folkens 2000.

Primary (Natural) Influences General accepted factors that can affect overall skeletal health, strength, architecture and disease.	**Secondary Influences** Additional ante-mortem influences that can cause bone loss as a secondary affect.	**Tertiary Influences** Post-mortem influences that can affect bone loss and its assessment.
Age	Immobilization	Taphonomy
Sex	Drug use (tobacco, alcohol, etc.)	Burial type +
Ethnicity	Infectious diseases	Diagenesis +
Diet & Nutrition (vitamins and minerals)*	Diet & Nutrition (vitamins and minerals)*	
Body weight and size	Metabolic diseases	
Genes	Trauma	
Hormones (estrogen)	Joint diseases	
Peak bone mass	Cultural aspects	
Mechanical loading		

* Diet and nutrition is an important basic function to health. It is both a primary and secondary influence.
+ aspects of taphonomy

2.2.2.2.1 Primary Influences

Age: One of the most accepted influences of bone loss is aging. As discussed above, there is a connection with increased age and increased bone loss due to inconsistent remodeling (Brickley and Ives 2008; Frost 2001). Males and females experience similar age related bone loss for both cortical and trabecular bone at approximately 20 – 30% (Brickley and Ives 2008).

Sex: In general females exhibit an increased risk of bone loss over males (Argwal 2003). Main factors that affect females are pregnancy, lactation, and menopause. Pregnancy and lactation can cause reversible BMD decrease of 5 – 7% in the proximal femur and vertebrae because of increased absorption of calcium from the skeleton (Brickley and Ives 2008).It has been suggested by Frinkelstein *et al.* (1992), that males with delayed puberty will have increased bone loss later in life. Males exhibit cortical bone loss of 5 – 10% each decade (Brickley and Ives 2008).

Ethnicity: Individuals of African descent exhibit less bone loss than those of Asian and/or Caucasian descent. This may be due to increased vitamin D absorption, parathyroid hormone production and more efficient calcium absorption and use in Africans (Brickley and Ives 2008).

Diet and Nutrition: Poor nutrition and obesity both cause bone loss. However, both of these extremes will be interrelated to different aspects. As stated above muscle strength plays a significant role in bone strength (Frost 2001). Individuals with poor nutrition still exert their bodies to normal and/or above normal levels of activity. Poor nutrition can sometimes be seen with reversible bone loss if an individual's diet improves. Obesity can cause immobilization effecting not only bone mass but also muscle strength. However, Steinchneider *et al*. (2003) found that BMC and BMD in the femoral neck of overweight females were higher than lean individuals. While the increased reading may be due to soft tissue interference, higher data reads for overweight individuals should be cautioned.

Current research provides information that we can assume applies to archaeological populations. As stated above, calcium is the main mineral found in the skeleton and adequate consumption is vital to reach peak bone mass and maintain healthy bone. Decreased intake and/or absorption of calcium increases osteoclast activity through hyperparathyroidism; insufficient protein causes the same chain of events (Brickley and Ives 2008). Lower protein intake also affects daily life causing fatigue, decreased muscle strength and subsequently increases risk of falling and fracture risk (Brickley and Ives 2008). Fatty acids such as omega-3's help calcium absorption; decreased consumption hinders absorption rates (Brickley and Ives 2008). Over consumption of fruits and vegetables has the potential to limit osteoclast activity (Brickley and Ives 2008). Insufficient vitamin C increase anemia risk causing decreased osteoblast activity and decreased osteon deposition during remodeling in load bearing peripheral elements (Brickley and Ives 2008). These nutritional factors are used to maintain extracellular fluid pH levels between 7.25 and 7.45 when increased in reversible bone loss (Brickley and Ives 2008).

The current research reviewed above is based on modern populations; archaeological diet consumption indices are complex and only provide generalized information such as C4 and C3 plant consumption categories (Larsen 1997). There are two main ways to detect poor nutrition in past populations. Chemical analysis such as stable carbon and nitrogen isotopes and trace elements can provide useful information about an individual's consumption profile (Larsen 1997; Roberts and Manchester 2007). The second is standard paleopathological assessment of an individual for lesions indicating a dietary deficiency. For example, the presence of porotic hyperostosis suggests that an individual had iron deficiency anemia at some point prior to death, or the presence of enamel hypoplasia, both highly correlated to nutritional stress during childhood (Larsen 1997; Roberts and Manchester 2007). As well, malnutrition weakens the immune system and subsequently leaves an individual more susceptible to disease (Roberts and Manchester 2007).

Body weight and size: Individuals that have stature and weight outside of the normal population average will have different bone mass because of their size. For example, Ibarhim *et al.* (2011) evaluated adolescent and adult Egyptain BMD and determined that adolescents with stunted growth and adults of short stature had lower bone mass.

Genes: Genetic coding dictates an individual's bone physiology through life. While external factors affect change in some ways, genetics are the backbone. Conditions such as a higher fracture susceptibility could be passed on from generation to generation (Agarwal 2008). "To date, no single straightforward genetic contribution to age-related osteoporosis has been identified (Brickley and Ives 2008, 157)."

Hormones: A decrease in estrogen during menopause in females increases bone remodeling with a 90% increase in osteoclast activity causing bone loss of 5 – 10% cortical and 20 – 30% trabecular (Brickley and Ives 2008). Not all women experience the same rates of menopausal bone loss and thus reach bone loss significant with fracture risk, osteoporosis (Brickely and Ives 2008). Sex hormones control overall trabecular structure in both females and male with male

trabecular thinning dictated by insulin-like growth factor 1 (Prevrhal *et al.* 2008). Because hormone levels are not determinable within archaeological material, modern studies must be relied upon. Current research indicates a high correlation between hormones and bone physiology (Bandeira *et al.* 2010; Meskek *et al.* 2010).

Peak bone mass: Inability to reach peak bone mass prior to remodeling increases bone loss later in life. It has been suggested that males who reach peak bone mass later in life, due to delayed puberty, tend to have less bone loss; a bias when comparing old adult males and females (Brickley and Ives 2008). However, this information is contradictory to earlier research in 1992 by Frinkelstein *et al.* who suggested similar rates of bone loss in male and female old adults when males experienced delayed peak bone mass later in life. Further research is needed to clarify this issue.

Mechanical loading: Physical activity dictates muscle and bone strength with excessive or insufficient exercise greatly affecting bone mass. Increased activity as an individual ages causes decreased osteoblast activity (Brickley and Ives 2008). Decreased activity as in individual ages increases fracture risk; an individual is more likely to fall because of decreased muscle strength (Brickley and Ives 2008). Excessive exercise can create hormonal imbalances that can lead to increased osteoclast activity causing lower bone mass such as seen in professional athletes (Brickley and Ives 2008).

2.2.2.2.2 Secondary Influences

Trauma: Ante-mortem trauma can first cause a loss of bone material and while healing will lead to bone gain as new bone is laid down to repair the injury site. Treatment methods of the traumatic injury will dictate repair functionality. For example, a femoral diaphysial fracture that is not set properly and heals can cause shortening of the affected limb.

Immobilization: Immobilization of a limb or whole body will cause a temporary decrease in bone mass in that location (Brickley and Ives 2008). However, long term immobilization of load-bearing elements can result in skeletal and muscle atrophy, osteoclast activity increases with disuse, such as seen with astronauts in

space (Brickley and Ives 2008). For example, if an individual experiences amputation of the tibia and fibula and does not put normal strain on the femur (through the help of a prosthesis, for example) then femoral bone loss will increase due to immobilization of the limb. The presence of partially healed fractures could suggest that the individual has a lower bone mass while individuals with healed fractures will most likely have a slightly higher bone mass from compensation of use of the opposite limb as an adaptive reaction to the bone.

Drug use: Extensive substance abuse such as smoking will causes a decrease in bone mass (Kamer *at el.* 2006). Young smoker's exhibit increased fracture risk (Tase *et al*. 2010) and older individuals also exhibit decreased calcium absorption (Krall and Dawson-Hughes 1999). Males and females who are heavy smokers present osteoporotic symptoms earlier in life than non-smokers (Kamer *at el.* 2006). The presence of stem pipe grooves in the dentition and pipe preservation as grave goods indicates smoking.

Infectious diseases: Infectious diseases can be determined by lesion presence and distribution. Infections can cause bone loss, bone gain, or a mix of both (Roberts and Manchester 2007). For example, osteomyelitis causes bone loss in the form of pitting and possible interior cavity formation *(ibid* 2007). Additionally, tuberculosis is diagnosable in archaeological material by the presence of sever vertebral collapse and Potts's disease. Lack of mobility due to inflammation caused by the disease is what causes localized increased bone loss and immobility (Brickley and Ives 2008).

Metabolic diseases: Metabolic disorders are characterized by conditions what are caused through the disruption of modeling and remodeling processes through cellular defects (Brickley and Ives 2008). Diseases such as rickets and osteomalacia are both caused by a vitamin D deficiency that causes bowing of the limb bones and decreased calcium absorption (Brickley and Ives 2008). Vitamin D deficiencies are more prevalent in regions with minimal sunlight. Of all the metabolic diseases, osteoporosis (advanced bone loss) is the most prevalent.

Cultural aspects: Culture characterizes a group's behavior, belief system, and traditions dictating all aspects of life such as occupation, social status, and

ritualistic behavior. A group/populations culture will affect all primary influences and some secondary influence (medical treatment and drug use) in different ways. Personal disposition will also affect these influences but for archaeological purposes an overall assemblages dynamic is normally grouped together and then sub-categorized as research continues.

Joint disease: Joint diseases can both increase and decrease bone mass. For example individual with degenerative joint disease (DJD) will have a higher bone density (Agarwal and Grynpas 2009). On the contrary, older individuals with joint degradation, such as that associated with rheumatoid arthritis, will have increased bone loss due to lack of movement within the joint and future permanent immobilization (Brickley and Ives 2008).

2.2.2.2.3 Tertiary influences

Taphonomy: After burial, a multitude of variables affect human remains. Temperature, humidity, soil type, microorganisms, and pH levels affect bone deterioration and subsequent preservation (White and Folkens 2000). Decomposition rates are influenced by taphonomy, burial type, and cultural practices prior to death.

Burial type: Open air burials are subject to more preditorial activity and disarticulation ultimately resulting in increased loss of skeletal elements. Coffin burials normally produce better preserved skeletons. However, different coffin types provided different protection rates for bone such as oak coffins causing better preservation and pine coffins with poorer preservation(Fiedler and Graw 2003). Cremation will only leave small bone fragments.

Diagenisis: Diagenesis is the destruction of bone on the microscopic level. Jackes *et al.* (2001) determined that cortical density is altered within the burial environment and bone microstructure preservation is complex. Microstructure deterioration is caused by bacteria, mainly *Clostridium histolyticum*, which alters bone by production the enzyme collagenase that digests collagen. Environmental pH also affects bone diagenisis rates. High pH levels decrease the rate of hydroxyapatite disintegration. Their research concluded that microbial destruction

can strongly alter bone microstructure. This factor should be kept in mind with density and bone geometric analysis on archaeological assemblages.

Bone physiology is affected by a multitude of interacting factors. Understanding the aspects that influence bone physiology (ante- and post-mortem) provides a better understanding of bone loss in the past. For example the presence of osteoporosis in a young individual indicates mal-nutrition (Roberts and Wakely 1992). However, this is not as simple as it sounds for ante-mortem and post-mortem factors need to be considered before bone loss assessment can be considered. Bone loss is only partly understood in modern populations, and even less so in archaeological assemblages, which are riddled with assumptions based on modern research. Caution must be taken when evaluating bone physiology of past peoples.

3 Materials

3.1 Middenbeemster Site

Over the summer of 2011, the Faculty of Archaeology at Leiden University and Hollandia Archaeology excavated approximately 450 individuals from a cemetery in Middenbeemster, Netherlands. The cemetery was in use from 1623 to 1866 AD. In addition to the recovery of skeletal material, historical records were found providing exact ages of death, sex and social status for many of the individuals.

3.1.1 Historical overview

The following historical overview is from the Netherlands Department of Conservation (1998). Between 1609 and 1613, the reclamation of Beemster Lake through draining and infilling, produced a manmade landscape divided into a geographical grid (fig 5).

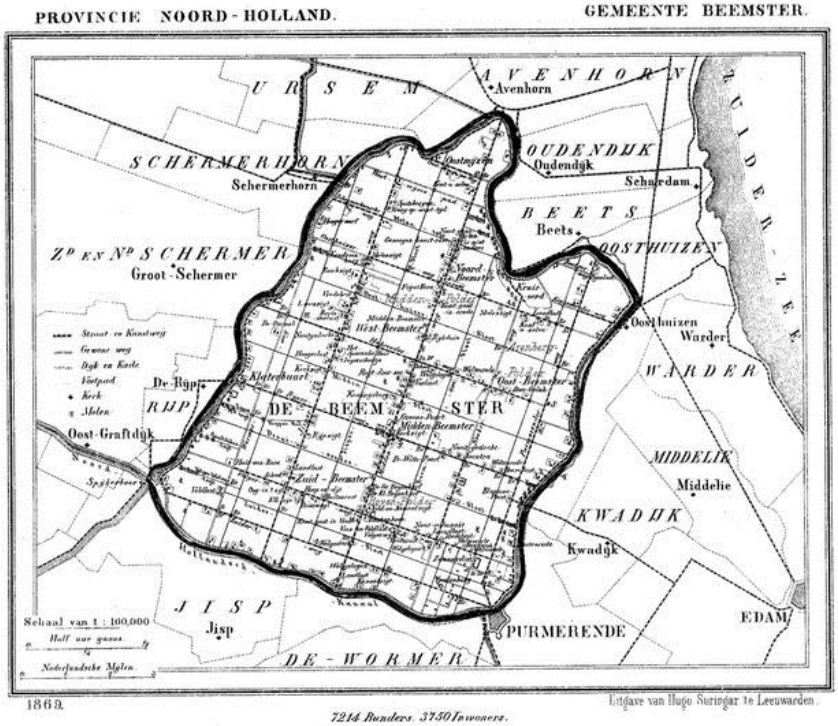

Figure 5: Historical map of the Beemster (http://www.humanosteoarchaeology.com/middenbeemster-2011.html)

The creation of Middenbeemster (located in North Holland) was supported by wealthy merchants as an investment opportunity to increase agricultural land and regulate flooding. Cereals, flax and rapeseeds were heavily cultivated at first

but eventually partials were converted into pastures with the increase of dairy production. Other occupations, based on historical records consist of, but are not limited to: merchants, tailors, cobblers, saddle makers, artists, carpenters, bakers, cargo delivers, water millers, mill bosses, housekeepers, gardeners, innkeepers, housewives, servants, law enforcement, and sailors. Many homes in Beemster were originally used as secondary homes for rich merchants.

Five churches were originally commissioned to be built but only one was constructed and in 1923, Hendrick de Keyser's design was completed. Located next to two major crossroads (Rijerwag and Middenwag) in the city's center, the church's adjoining cemetery was used until 1866. Individuals interned here, and within the church, consist of local inhabitants who were born and raised in the Beemster. As with the surrounding town, the cemetery was also organized in a grid pattern. Surviving burial records dating back to 1829 provide names, age at death, occupation and burial location. However, the clear organization presented in the records is not constant with that found during excavation. Wooden coffins were stacked and often overlapped, individuals were interned between designated rows, and the active removal and relocation of individuals elsewhere in the cemetery was done to make space for new burials. A new cemetery was designated in 1866 on the outskirts of Middenbeemster and is still in use today. The Beemster was designated a World's Heritage site by UNESCO in 1999.

3.2 Historical Records

Parish records are available for some individuals and provide information about age at death, sex, and who paid for individual burial plots. During this time period, individuals paid 30 gilders for a plot if they were wealthy. Poor individuals were buried for free. This division of payment provides us with an idea about social status. However, due to the fact that plots were rented (individuals were removed, new individuals replaced them) and individuals placed between known grave plots, the records are not coherent and are still in the process of decipherment. Because of this, the historical records were not completely available for use in this thesis and were not included. Decipherment of the archival records is still underway.

3.3 Element Selection

Two bones from 51 individuals (26 males and 25 females) were selected for BMC, BMD, and trabecular architecture determination consisting of a load bearing bone, the femur, and a non-load bearing bone, the humerus. Both elements were taken from the left side. If the left was extensively damaged or unavailable, the right side was used. Individual's ages range from 18 to 50+ years. The femur was chosen because it is mostly likely to show osteoporosis and thus standardized methodologies have been established to determine age related bone loss and has a high fracture rate in the elderly (Adams 2008). The humerus was chosen because current research indicates that its fracture rate is similar to that of the femur when pertaining to age related bone loss (Tingart *et al*. 2003b). Among the loading and no-loading skeletal elements, the femur and humerus were selected because of their loading and non-loading aspects. It should be noted that male individual MB11S497V1059, only had both femura selected and no humerus in order to evaluate the effects of a completely healed spiral fracture of the left tibia and fibula. Table 3 lists the major pathological condition seen within this sample that are associated with bone loss (either through primary or secondary influences). A complete list of conditions can be found in the material catalogue in Appendix A.

Table 3: Pathological conditions in the selected Middenbeemster sample associated with bone loss.

Pathological conditions with a known bone loss component seen in the Middenbeemster assemblage.
Slight Scoliosis
Micoporosity
Minor Cribrial orbitalia
Server Osteoarthritis
Rickets
Osteomalasia
Achondroplasia
Trauma: Healed spiral fracture

4 Methods

This chapter covers the technical aspects of skeletal analysis and QCT and DEXA assessment methodologies of bone loss. The relevance of age-at-death, sex, stature, body mass and pathology are discussed above in 2.2.2.2 non-mechanical influences.

4.1 Skeletal Analysis

Individuals were analyzed to determine age-at-death, sex, stature, body mass and pathology. The material catalog in Appendix A lists these details for each individual. Analysis was performed in the Osteoarchaeology Laboratory at Leiden University by the Osteoarchaeology MSc students under the supervision of Dr. Andrea Waters-Rist.

4.1.1 Age-at-death

Age-at-death was determined through the analysis of dental attrition (Maat 2001), auricular surface morphology (Burkberry and Chamberlin 2002), suture closure (Meindl and Lovejoy 1985), pubic symphysis (Brooks and Suchey 1990), and sternal rib end morphology (Işcan *et al.* 1984). Individuals were placed into the osteological age categories of early young adult (EYA) (18-25 years), late young adult (LYA) (26-34 years), middle adult (MA) (35-49 years), or old adult (OA) (50+ years). If it was possible to determine a smaller age range within a category, a side note was made and added to the osteological category.

4.1.2 Sex

Sex determination was based on the Workshop of European Anthropologists (WEA) (1980) and Buikstra and Ubelaker (1994). The WEA (1980) method is a weighted scoring system of cranial, mandibular and pelvic traits. Only adult individuals can be sexed. Traits were scored as female, possible female, indeterminate, possible male, or male. Each score was then calculated based on its degree of sexualisation weighted as 3, 2 or 1. The cranium, mandible and pelvis scores were calculated separately. Pelvic scores are more heavily weighted because the pelvis has the most pronounced sexual dimorphism. Additional post-cranial traits consisted of measurements determined to be male, female or indeterminate. A final sex estimate was based on the scores of the

cranium, mandible, pelvis and post-cranial measurements. Possible males were incorporated with males and possible females were incorporated with females for this study.

4.1.3 Stature

Stature was determined for each individual using Trotter's 1970 equations for white males and females. Maximum length of the left femur and length of the left tibia were obtained using an osteometric board. The following equations were used to determine stature:

Male	1.30 (Fem + Tib) + 63.29	SD ± 2.99
Female	1.39 (Fem + Tib) + 53.20	SD ± 3.55

Stature of individual MB11S428V0945 was calculated using total anatomical length because of achrondroplasia.

4.1.4 Body Mass

Body mass (BM) in kilograms for each individual was obtained through the following equations (Pomeroy and Stock 2012):

BM = 2.2393 x FHD – 39.9 (McHenry 1992)

BM = 2.2683 x FHD – 36.5 (Grine *et al.* 1995)

BM = 2.7413 x FHD – 54.9 (Ruff *et al.* 1991) – males only

BM = 2.426 x FHD – 35.1 (Ruff *et al.* 1991) - females only

that are based on FHD (maximum femoral head diameter) in millimeters. The equations (above), designed for specific population types: "pygmy" (McHenry 1992), exceptionally large (Grine *et al.* 1995), and modern white from the United States (Ruff *et al.* 1991) decreased by 10 percent for adiposity (Nikita *et al.* 2011), were averaged. This provides an accurate assessment for populations that fall within the normal range, which are not exceptionally small or large (Pomeroy and Stock 2012).

4.1.5 Pathology

Pathology observation was based on macroscopic examination of each skeletal element for every individual. The most commonly observed lesions were vertebral lipping, Schmorl's nodes, osteoarthritis, osteomalacia, dental calculus

and periodontal bone loss. A list of pathological conditions associated with each individual can be found in Appendix A.

4.2 Assessing Bone Loss

The methodologies to determine the presence of age-related bone loss in archaeological material are based on current medical standards. Comparisons to other populations was not undertaken because of the addition of an increasing number of factors that pertain to a specific burial location, culture and past life ways. In essence, comparing an agricultural population to that obtained from Middenbeemster is like comparing apple to oranges unless it was to another Dutch population which to my knowledge is not possible at this time.

4.2.1 QCT

The femur and humerus from fifty-one individuals were scanned with a Philips Brilliance 64 CT scanner at the Amsterdam Medical Center, Amsterdam, Netherlands. Elements were placed on a flat board as close to anatomical position as possible and scanned; no soft tissue substitute was used (Tingart *et al.* 2003b). Scans were taken at 1 mm increments, 120 kv, and a 250.0 mm field of view (FOV). No space was left in-between slices providing a complete 3D image of each bone after rendering. A calibration phantom (Image Analysis System) of calcium hydroxyapatite concentrations (fat, 0, 50, 100 and 200 mg/cm^3) that is set in water-equivalent plastic was included in each scan to determine Hounsfield Units (HU)/BMC (van Rijn and van Kuijk 2008). After scans were rendered, the PACS program was used for skeletal analysis. Each bone was manually positioned into anatomical position (fig 6) using Ruff's (2002) x,y,z positioning technique. Additional rotations were made to obtain femoral neck measurements based on the neck coordinate system described by Kang *et al.* 2005.

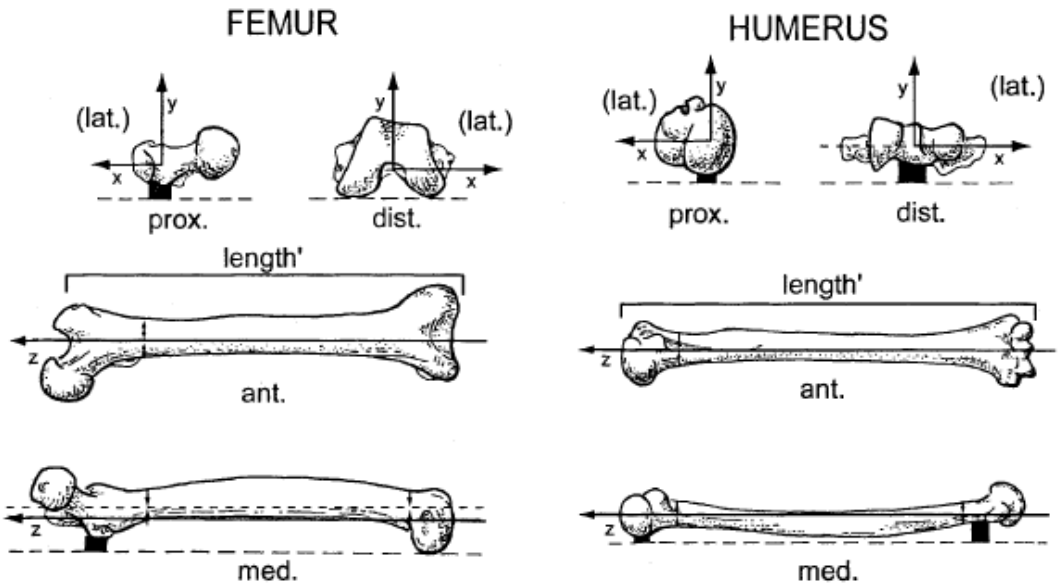

Figure 6: Femoral and humeral 3D anatomical positioning (Ruff 2002, 338)

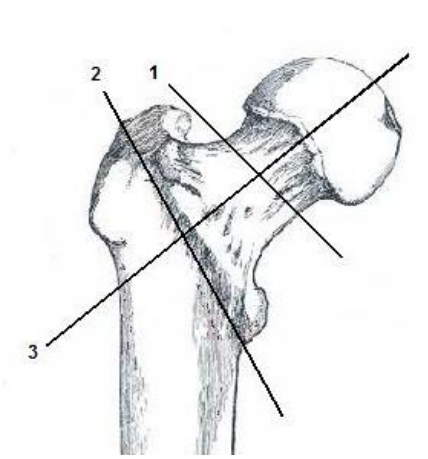

Figure 7: QCT femur slice locations (modified image after Gray 1918, http://www.bartleby.com/107/illus244.html)

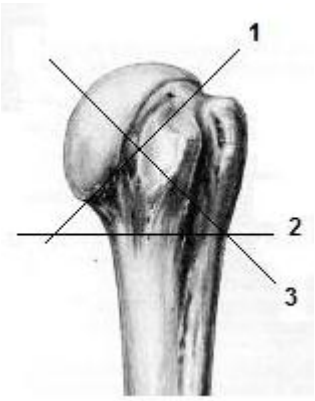

Figure 8: QCT humerus slice locations (modified image after Gray 1918, http://www.bartleby.com/107/illus207.html)

Four 1.0 mm^3 cortical bone densities were taken, within a 5.0 mm space, for each slice on a x, y axis that were averaged. A 5.0 mm space was used to provide a better chance of obtaining a density of pure cortical bone and to decrease error rates of obtaining the exact 1.0 mm^3 reading at the same location on

each bone. Additionally, this was done to mineralize the risk of obtaining a highly negative density reading. Initial test data selection produced highly negative values for trabecular bone HU readings which were determined to an effect of scanning the elements in air. Current preprogramming registers air as having a HU value of -1000 with tissue and bone having positive values. In light of this, it was decided to average four 1.0 mm3 reading to calculate cortical HU and subsequently cortical $_{v}$BMD because bone should have a positive reading (van Rijn and van Kuijk 2008).

ImageJ (http://rsb.info.nih.gov/ij/) plugin, BoneJ (Doube *et al.* 2010), was used to determine area (cm^2), volume (cm^3), trabecular thickness (Dougherty and Kumzelmaan 2007; Hidebrand and Rüegsegger 1997), and trabecular connectivity (Odgaard and Gundersen 1993; Toriwaki and Yonekura 2002) of each slice. This free online software package was used because factory specific programming for this type of analysis was not available at the Amsterdam Medical Center, only a few facilities in the world have the proper programming to produce reliable readings (Endelke *et al.* 2008). Table 4 provides a list of data type analyses details that were derived from BoneJ. The inclusion of trabecular volume, thickness/spacing and connectivity should not be underestimated when reviewing QCT $_{v}$BMD data. It is the hope that one or all of these data types will help clarify age related bone loss within this thesis sample population.

Table4 : QCT Processed Data abbreviations.

Data type	Abbreviation	Units	Description	Citation
Area	CA	cm^2	Cortical area only	Doube et al (2010)
	TA	cm^2	Trabecular area only	
	ROIA	cm^2	Total slice area	
Volume	BV	cm^3	Bone volume*	Doube et al (2010)
	TV	cm^3	Total volume*	
	BV/TV	cm^3	Bone volume function*	
	CAV	cm^3	Cortical bone volume+	
Trabecular thickness	**Tb.Th mean**	cm^3	Trabecular thickness mean	Dougherty and Kunzelmaan (2007); Hildebrand and Rüegsegger (1997)
	Tb.Th SD	cm^3	Trabecular thickness standard deviation	
	Tb.Th max	cm^3	Trabecular thickness max	
	Tb.Sp mean	cm^3	Trabecular spacing mean	
	Tb.Sp. SD	cm^3	Trabecular spacing standard deviation	
	Tb.Sp. max	cm^3	Trabecular spacing max	
Connectivity	Euler ch.		Eular characterististic of the sample as through floating in space (X)*	Odgaard and Gundersen (1993); Toriwaki and Yonekura (2002)
	Δ(X)		The bone sample's contribution to the Euler characteristic of the bone to which it is connected*	
	Conn	#	The connectivity of the image (~ number of trabeculae)*	
	Conn.D	cm^3	Connectivity density (~ number of trabeculae per unit volume)*	

*Trabecular bone only
+ cortical bone volume was calculated by the author of this thesis.
Bold abbreviations indicated data types used in bone loss assessment for this study.

4.2.2 DEXA

The femur and humerus were scanned with a Hologic Discovery A QDR Series Scanner (S/N 85634) at the Amsterdam Medical Center, Amsterdam, Netherlands. Each element was scanned individually under the supervision of Ehsan Hemayat, at 70 to 140kVp. Femora were placed on 15 cm of dry rice (Mays *et al.* 1998), positioned so that the femoral head and neck were flat so that the lesser tuberosity was still visible on the scan. Humeri were placed on 10 cm (Tingart *et al.* 2003a, Tingart *et al.* 2003b) of rice so that the bicipital groove was parallel to the central axis of the scanner and the greater tubercle and lesser

tubercle could be easily identified on each scan. Both the femur and humerus were scanned with the preset scan type of either left or right hip depending on which side the element was from. Analysis was preformed with Hologisc software version 13.3:3. Femora data were obtained by standard programming (Boussen *et al.* 2006). Humari data were gathered using subregion array regions of interest based on Tingart *et al*.'s (2003a) analysis of cadaver humeri.

BMC and BMD for the femur were obtained for the following three areas (fig 9):

1) $DEXA_{Fneck}$ - femoral neck, (rectangular box encompassing approximately the total neck region)
2) $DEXA_{Ftroch}$ - trochantar cross section originating form the lateral interaction point of the DXA_{Fneck} and DXA_{Finter} through the middle of the trocanters.
3) $DEXA_{Finter}$ - medial lateral midline through the femoral head, neck and trochantar area.

Humeral BMC and BMD scores were obtained from three areas (fig 10):

1) $DEXA_{Hhead}$ - humeral head including surgical neck ending approximately 1cm below head
2) $DEXA_{Hgtub}$ - greater tuberosity excluding bicipital groove
3) $DEXA_{Hltub}$ - lesser tuberosity and humeral head, excluding bicipital groove.

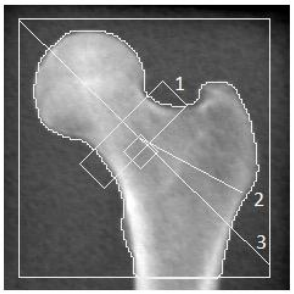

Figure 9: DEXA femur data collections sites. Image of individual MB11S059V0133

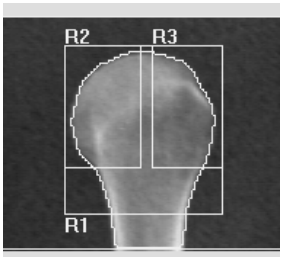

Figure 10: DEXA humerus data collection sites. Image of individual MB11S059V0133

4.3 Statistics

Statistical analysis was performed with SPSS version 19. Data normality was assessed with a Levene's test. If normality was not validated ($p \leq 0.05$), non-parametric statistics (Mann-Whitney U test) were used because equal variances between groups could not be assumed. Sex differences were determined using an individual sample t- differences were assessed with Oneway ANOVA's, using Tukey as a post-hoc test in case any significant results were obtained. Post-hoc test Tukey uses standard deviations to correct for type 1 error, a true false positive.

5 Results

This chapter will be broken down as follows. Data is first broken down per data type; bone mineral concentration, bone mineral density, trabecular bone volume, trabecular thickness, trabecular spacing, connectivity and connectivity density. Within each data type, each element is discussed with male and female data reviewed separately for all slices in relation to age, concluding with a "summary" review of what the data is saying. Then a cross examination between data types, machines, slices, age and sex will be presented. A summary of all results will be presented at the end.

Of the approximately 450 individuals excavated from Middenbeemster, 102 elements (femur n = 52, humerus n = 50) from a sub-sample of fifty-one individuals (females n = 25; males n = 26) were scanned with both QCT and DEXA for a total of 204 scans. Usable QCT data was obtained from 40 femura and 39 humeri, and DEXA data consists of 48 femura and 50 humeri. All together, a total of 2178 data points (QCT n = 1590; DEXA n = 588) were obtained. The table 5 below details the number of data points per slice for each machine.

Table 5: Total number of femoral and humeral data points per data type, slice and machine.

Data type	QCT_{Fneck}	QCT_{Ftroch}	QCT_{Fcross}	QCT_{HAneck}	QCT_{HSneck}	QCT_{Hcross}	$DEXA_{Fneck}$	$DEXA_{Ftroch}$	$DEXA_{Finter}$	$DEXA_{Hhead}$	$DEXA_{Hgtub}$	$DEXA_{Hltub}$	**Total**
Bone mineral concentration (BMC)*	40	39	40	37	37	37	48	48	48	50	50	50	524
Bone mineral density (BMD)*	40	39	40	37	37	37	48	48	48	50	50	50	524
Trabecular bone volume (BV/TV)	38	38	39	37	37	37	n/a	n/a	n/a	n/a	n/a	n/a	226
Trabecular thickness (Tb.Th mean)	38	38	39	37	37	37	n/a	n/a	n/a	n/a	n/a	n/a	226
Trabecular spacing (Tb.Sp mean)	38	38	39	37	37	37	n/a	n/a	n/a	n/a	n/a	n/a	226
Connectivity (Conn)	38	38	39	37	37	37	n/a	n/a	n/a	n/a	n/a	n/a	226
Connectivity density (Conn.D)	38	38	39	37	37	37	n/a	n/a	n/a	n/a	n/a	n/a	226
Total	270	268	275	259	259	259	96	96	96	100	100	100	***2178***

*BMC and BMD readings for QCT were calculated for cortical bone only. The BMC and BMD for DEXA calculations are a combination of both cortical and trabecular bone.

5.1 Bone Mineral Concentration (BMC) (fig 11, 12, 13, and 14)

Bone mineral concentration for DEXA was calculated for combined cortical and trabecular bone and is expressed in grams. QCT bone mineral concentration was calculated for cortical bone only and is expressed in Hounsfeld units.

***5.1.1 Femur*:**

Female bone mineral concentration (BMC): All female DEXA slices $DEXA_{Fneck}$, $DEXA_{Ftroch}$, and $DEXA_{Finter}$ show a slight BMC increase from early young adult (EYA) to late young adult (LYA), a decrease from LYA to middle adult (MA), and an increase from MA to old adult (OA). Female $DEXA_{Fneck}$ and $DEXA_{Ftroch}$ have a similar pattern even though $DEXA_{Ftroch}$'s overall BMC per age is higher. $DEXA_{Finter}$ presents a much larger increase in BMC from MA to OA. All female QCT slices ($QCT_{Fneck,}$ QCT_{Ftroch}, and $QCT_{Fcross)}$ indicate an average BMC increase from EYA to LYA with a slight decrease from LYA to MA. There is a BMC decrease from MA to OA for QCT_{Fneck} and QCT_{Ftroch} but an increase for $QCT_{Fcross.}$

Male bone mineral concentration (BMC): For the males, there is a small decrease from early young adult (EYA) to late young adult (LYA) BMC in $DEXA_{Fneck}$ and $DEXA_{Ftroch}$ but an increase between these ages in slice $DEXA_{Finter}$. $DEXA_{Fneck}$ shows a plateau from LYA to middle adult (MA) while $DEXA_{Ftroch}$ and $DEXA_{Finter}$ indicate a decrease in average BMC. MA to old adult (OA) average BMC's decrease slightly for $DEXA_{Fneck}$, plateau for $DEXA_{Ftroch}$, and increase in slice $DEXA_{Finter}$. Male BMC increases from EYA to MA and decreases from MA to OA for QCT_{Fneck} and QCT_{Ftroch}. However, for QCT_{Fcross} there is minimal BMC increase from EYA to LYA, a large increase from LYA to MA with a subsequently large decrease from MA to OA.

Femoral BMC statistical analyses: Males have a significantly higher BMC than females for all femoral DEXA scans; $DEXA_{Fneck}$ ($t = 3.404$; $df = 46$; $p = 0.001$), $DEXA_{Ftroch}$ ($t = 3.077$; $df = 46$; $p = 0.004$) and $DEXA_{Finter}$ ($t = 3.930$; $df = 46$; $p = 0.000$). No significant difference was found between male and female QCT femoral slices and all age-related DEXA and QCT BMC (table 6). Male

only age-related BMC in $DEXA_{Fneck}$ is statistically significant (f = 3.398; df = 3; p=0.038) There is no statistical difference for male only age-related BMC for $DEXA_{Ftroch}$ and $DEXA_{Finter}$ (table 6). Male only QCT, female only DEXA, and female only QCT were not analyzed statistically for BMC because one or more age brackets had only one individual when subdivided by sex.

5.1.2 Humerus*:*

Female bone mineral concentration (BMC): Female average BMC for $DEXA_{Hhead}$ shows a steady decrease from early young adult (EYA) to old adult (OA). There is a plateau from EYA to late young adult (LYA) in $DEXA_{Hgtub}$ with a decrease from middle adult (MA) to OA. $DEXA_{Hltub}$ indicates an average BMD plateau from EYA to OA. QCT_{HAneck} female average BMC increases from EYA to LYA, decreases from LYA to MA, and increases again from MA to OA. Both QCT_{HSneck} and QCT_{Hcross} have a BMC increase from MA to OA. However, QCT_{HSneck} has an increase from EYA to LYA and a decrease to MA, while QCT_{Hcross} plateaus from EYA to LYA and slightly increases from LYA to MA.

Male bone mineral concentration (BMC): Male average BMC steadily increases from early young adult (EYA) to old adult (OA) for $DEXA_{Hhead}$. $DEXA_{Hgtub}$ plateaus for EYA to late young adult (LYA) followed by a slight decrease to middle adult (MA) and an increase to OA. $DEXA_{Hltub}$ has a steady increase from EYA to MA and a decrease from MA to OA. Male QCT_{HAneck} average BMC increases from EYA to OA; this is the same pattern as male $DEXA_{Hhead}$ BMC averages. QCT_{HSneck} indicates an increase from EYA to MA and then a decrease from MA to OA. QCT_{Hcross} plateaus from MA to OA; there is an increase from EYA to LYA and a slight decrease from LYA to MA.

Humeral BMC statistical analyses: Statistically, DEXA male humeri have a higher BMC than female humeri; $DEXA_{Hhead}$ (t = 5.736, df = 48; p = 0.000), $DEXA_{Hgtub}$ (z = -3.444; p = 0.000), and $DEXA_{Hltub}$ (z = -4.677; p = 0.000). Additionally, male BMC in QCT_{Hcross} is higher than females (t = 2.345; df = 34; p = 0.025). There is no significant difference between the sexes for QCT_{HSneck} and QCT_{HAneck} nor is there any age-related BMC significant difference for all humeral DEXA and QCT slices (table 6). Additionally, no significant differences are

present for male only humeral DEXA slices. Male only QCT, female only DEXA, and female only QCT were not analyzed statistically for BMC because one or more age brackets had only one individual when subdivided by sex.

5.1.3 Summary:

DEXA statistically determined that males have a higher BMC than females for both the femur and humerus. $DEXA_{Fneck}$ male only average BMC was statistically higher when compared to the age groups, however all other slices were not able to be analyzed because of a small sample size; some age groups had only one individual. From early young adult to old adult, $DEXA_{Hhead}$ BMC steadily decreases in females but increases in males. Both males and females have a much higher $DEXA_{Finter}$ average BMC for each age group than $DEXA_{Fneck}$ and $DEXA_{Ftroch}$ which may be due to the larger overall slice area for the $DEXA_{Finter}$. The old adult BMC average for QCT_{Fcross} is increased and is similar for males and females (male 27.92g; female 28.38g). Female $DEXA_{Fneck}$ and $DEXA_{Ftroch}$ have a similar pattern change between age groups.

Table 6: Bone mineral concentration (BMC) sex and age statistical data.

	Sex-related BMC			**Age-related BMC**		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	0,252	38	0,602	1,435	3	0,249
QCT_{Ftroch}	0,467	36	0,643	1,179	3	0,332
QCT_{Fcross}	-0,472	38	0,640	0,853	3	0,474
$DEXA_{Fneck}$	3,402	46	**0,001**	2,191	3	0,103
$DEXA_{Ftroch}$	3,077	46	**0,004**	1,531	3	0,220
$DEXA_{Finter}$	3,930	46	**0,000**	1,712	3	0,178
QCT_{HAneck}	0,820	34	0,418	0,973	3	0,418
QCT_{HSneck}	-1,360	34	0,183	0,709	3	0,554
QCT_{Hcross}	2,345	34	**0,025**	0,426	3	0,736
$DEXA_{Hhead}$	5,736	48	**0,000**	0,531	3	0,663
$DEXA_{Hgtub}$	-3,444	48	**0,001***	0,580	3	0,631
$DEXA_{Hltub}$	-4,677	48	**0,000***	0,152	3	0,928

* Mann-Whitney U test z value

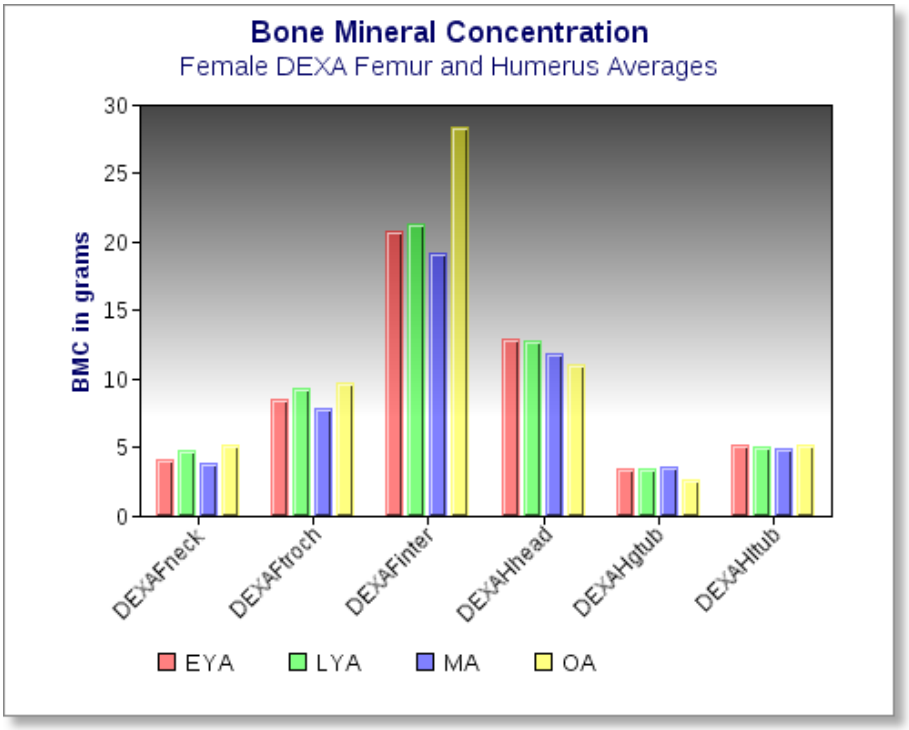

Figure 11: Female DEXA average bone mineral concentration (BMC) for femoral and humeral slices: age to BMC (in grams) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

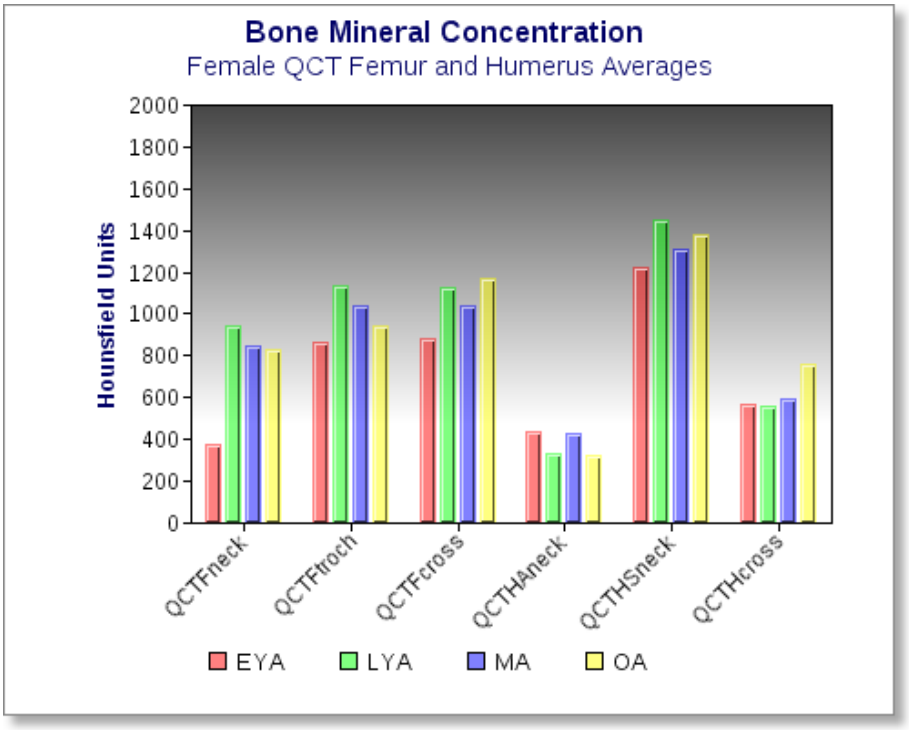

Figure 12: Female QCT average bone mineral concentration (BMC) for femoral and humeral slices: age to Hounsfiled Unit comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

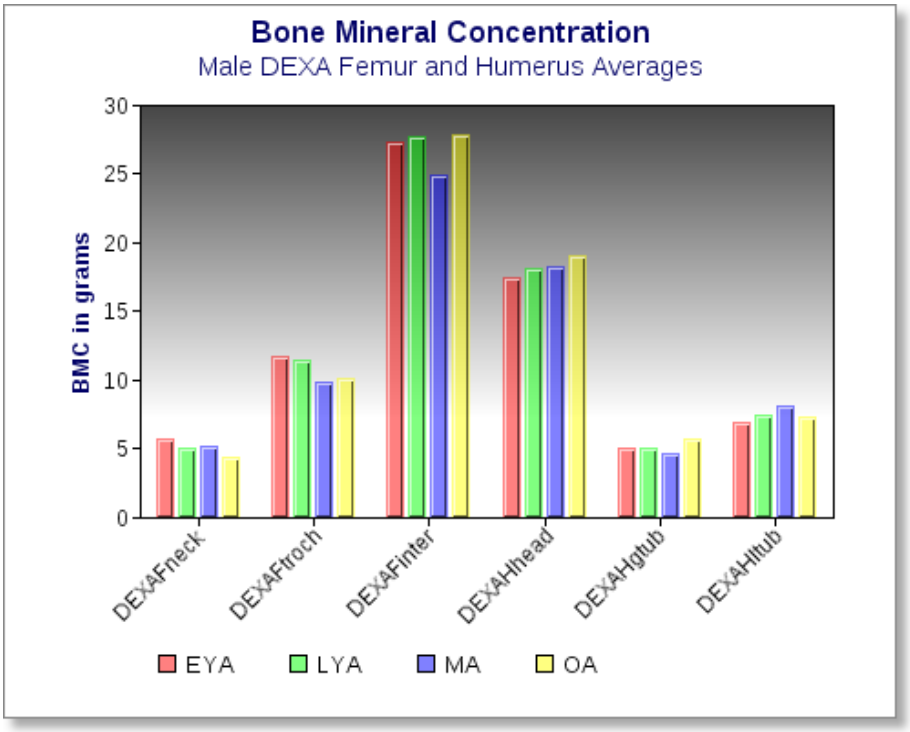

Figure 13: Male DEXA average bone mineral concentration (BMC) for femoral and humeral slices: age to BMC (in grams) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

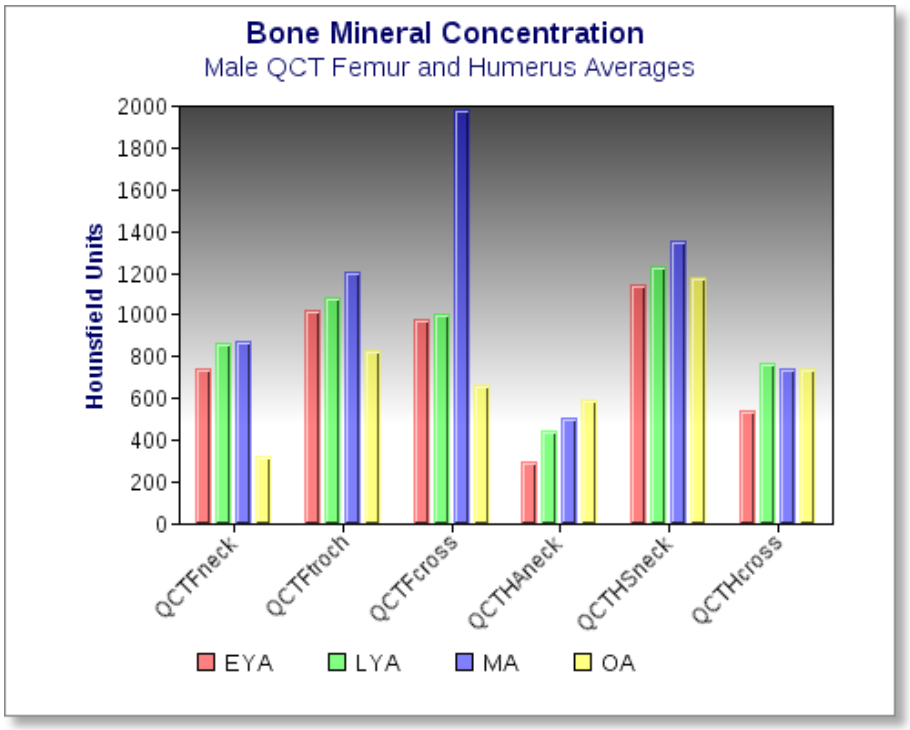

Figure 14: Male QCT average bone mineral concentration (BMC) for femoral and humeral slices: age to Hounsfield Units comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.2 Bone Mineral Density (BMD) (fig 15, 16, 17, and 18)

Bone mineral density is calculated as grams over regions of interest (see chapter 1). DEXA bone mineral density was calculated for combined cortical and trabecular bone. It is expressed as g/cm^2 and abbreviated at $_aBMD$ (areal bone mineral density). QCT bone mineral density was calculated for cortical bone only. It is expressed as g/cm^3 and abbreviated as $_vBMD$ (volumatic bone mineral density).

5.2.1 Femur:

Female bone mineral density (BMD): There is a slight increase in average $_aBMD$ from early young adult (EYA) to late young adult (LYA) with a decrease in middle adult (MA) females in all three DEXA slices ($DEXA_{Fneck}$, $DEXA_{Ftroch}$, $DEXA_{Finter}$). There is an increase in average $_aBMD$ in MA to old adult (OA) females which was unexpected. All female QCT scans (QCT_{Fneck}, QCT_{Ftroch} and QCT_{Fcross}) indicate a dramatic decrease in average $_vBMD$ from MA to OA. An increase is present from EYA to MA for QCT_{Fneck}. QCT_{Ftroch} has an increase from EYA to LYA which plateaus at MA. The female QCT_{Fcross} slice indicates a plateau in average $_vBMD$ from EYA to MA. There is no clear age-related pattern present for female average BMD between DEXA and QCT (fig 15 and 16). However, DEXA scans (fig 15) present a similar pattern for all three female femoral slices.

Male bone mineral density (BMD): There is a decrease in average $_aBMD$ from early young adult (EYA) to late young adult (LYA) for all DEXA slices as well as a decrease from middle adult (MA) to old adult (OA) for $DEXA_{Fneck}$ and $DEXA_{Finter}$; MA to OA increases for $DEXA_{Ftroch}$. However, there is an increase for LYA to MA for $DEXA_{Fneck}$ with a plateau present for $DEXA_{Ftroch}$ and $DEXA_{Finter.}$ Male QCT slice patterns are the same as those for females except that MA to OA slices do not decrease as dramatically as in the females, with MA to OA decreasing in QCT_{Fneck}, slightly increasing in QCT_{Ftroch} and decreasing in QCT_{Fcross} (fig 16 and 18).

Femoral BMD statistical analyses: There is no statistical difference between males and female for femoral BMD (table 7). However, age-based

femoral $_{v}$BMD averages (early young adult to old adult) do differ significantly for QCT: QCT_{Fneck} (f = 9.876; p = 0.000), QCT_{Ftroch} (f = 7.230; p = 0.001), and QCT_{Fcross} (f = 7.672; p = 0.000). There is no significant difference for age-based femoral DEXA $_{a}$BMD (table 7). Statistical analysis indicated a strong difference in male only age-related $_{a}$BMD between early young adult and late young adult and early young adult and old adult, for $DEXA_{Fneck}$ (f = 5.767; p = 0.005). There is no statistical difference for male only age-related $_{a}$BMD for $DEXA_{Ftroch}$ and $DEXA_{Finter}$ (table 7). Male only QCT, female only DEXA, and female only QCT were not analyzed statistically for BMD because one or more age brackets had only one individual when subdivided by sex.

5.2.2 Humerus:

Female bone mineral density (BMD): All female DEXA slices ($DEXA_{Hhead}$, $DEXA_{Hgtub}$, and $DEXA_{Hltub}$) indicate there is a steadily decreasing average $_{a}$BMD from early young adult (EYA) to old adult (OA). Again, all three DEXA female humeral slices present a similar pattern (fig 15). The QCT data is scattered. As with the female QCT femoral scans, a dramatic decrease is seen from middle adult (MA) to OA for QCT_{HAneck}, QCT_{HSneck} and QCT_{Hcross}. Female QCT_{HAneck} shows an increase from late young adult (LYA) to MA (no EYA data was available for this slice), QCT_{HSneck} presents an increase from EYA to LYA but a decrease to MA, while QCT_{Hcross} indicates a steady average $_{v}$BMD from EYA to MA. As with the femur, there is no clear age-related pattern present for female average BMD between DEXA and QCT (fig 15 and 16).

Male bone mineral density (BMD): Male DEXA humeral scans indicate a plateau in early young adult (EYA) to middle adult (MA) average $_{a}$BMD for $DEXA_{Hhead}$ and $DEXA_{Hltub}$. $DEXA_{Hgtub}$ EYA to late young adult (LYA) $_{a}$BMD plateaus but then dramatically increases from LYA to MA. All three male DEXA humeral slices show decreased $_{a}$BMD from MA to old adult (OA). QCT_{HAneck} and QCT_{HSneck} indicate an increase of average $_{v}$BMD from EYA to OA with QCT_{Hcross} presenting a similar pattern as $DEXA_{Hltub}$ except for an increase from EYA to LYA.

Humeral BMD statistical analyses: Statistically, males have a higher $_{a}$BMD than females for $DEXA_{Hhead}$ ($t = 2.262$; $df = 48$; $p = 0.017$) and $DEXA_{Hltub}$ ($t = 2.666$; $p = 0.010$). There is no statistical difference between males and females for all other humeral DEXA and QCT slices (table 7). An age-based significant difference can be seen in QCT humeral $_{v}$BMD slices QCT_{HSneck} ($f = 9.016$; $p = 0.000$) and QCT_{Hcross} ($f = 9.981$; $p = 0.000$) pertaining to age (early young adult and old adult). No statistical difference is present for all other humeral DEXA and QCT slices (table 7). Additionally, no significant differences are present for male only humeral DEXA slices. Male only QCT, female only DEXA, and female only QCT were not analyzed statistically for BMD because one or more age brackets had only one individual when subdivided by sex.

5.2.3 Summary:

Female humeral $_{a}$BMD decreases steadily from early young adult to old adult while female femoral DEXA $_{a}$BMD decreases from late young adult to middle adult with a subsequent increase to old adult for all three DEXA slices. QCT data show a statistically significant decreased average $_{v}$BMD, from early young adult to old adult, for the sample as a whole, except for the humeral anatomical neck, QCT_{HAneck}. However, while statistically this statement is true, the result itself may be a false positive. The female QCT $_{v}$BMD old adult reading is highly negative (fig 16), which could have caused the statistical analysis to think that there was a statistically significant increase. In figures 17 and 18, a similarity can be seen between the overall pattern of male age-related changes infemoral QCT_{Fcross} and humeral $DEXA_{Hhead}$ and $DEXA_{Hltub}$. As noted above, it must be remembered that DEXA measures areal density of both cortical and trabecular bone combined while QCT measures cortical only volumatic density.

Table 7: Bone mineral density (BMD) sex and age statistical data.

	Sex-related BMD			Age-related BMD		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	0,252	37	0,803	9,876	3	**0,000**
QCT_{Ftroch}	0,417	36	0,679	7,230	3	**0,001**
QCT_{Fcross}	-0,191	38	0,850	7,672	3	**0,000**
$DEXA_{Fneck}$	0,977	46	0,334	2,848	3	**0,048**
$DEXA_{Ftroch}$	0,291	46	0,772	2,507	3	0,071
$DEXA_{Finter}$	-0,095	46	0,925	1,754	3	0,170
QCT_{HAneck}	0,761	34	0,452	1,426	3	0,253
QCT_{HSneck}	0,429	34	0,671	9,016	3	**0,000**
QCT_{Hcross}	1,078	34	0,289	9,981	3	**0,000**
$DEXA_{Hhead}$	2,262	48	**0,017**	0,578	3	0,632
$DEXA_{Hgtub}$	1,349	48	0,184	0,444	3	0,723
$DEXA_{Hltub}$	2,666	48	**0,010**	0,739	3	0,534

* Mann-Whitney U test z value

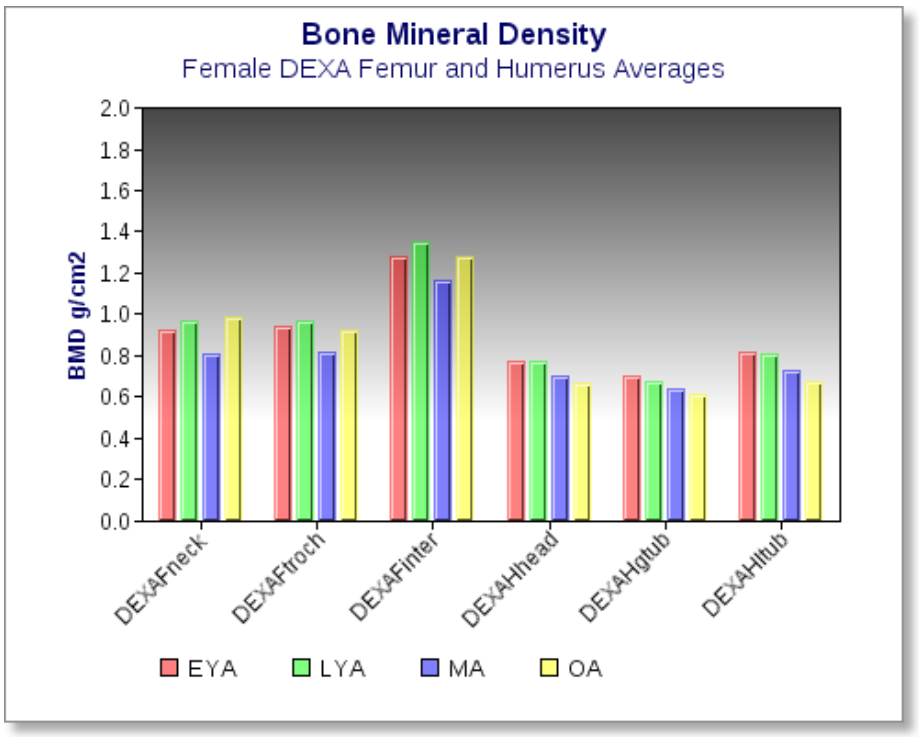

Figure 15: Female DEXA average bone mineral density (BMD) for femoral and humeral slices: age to areal BMD (g/cm^2) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

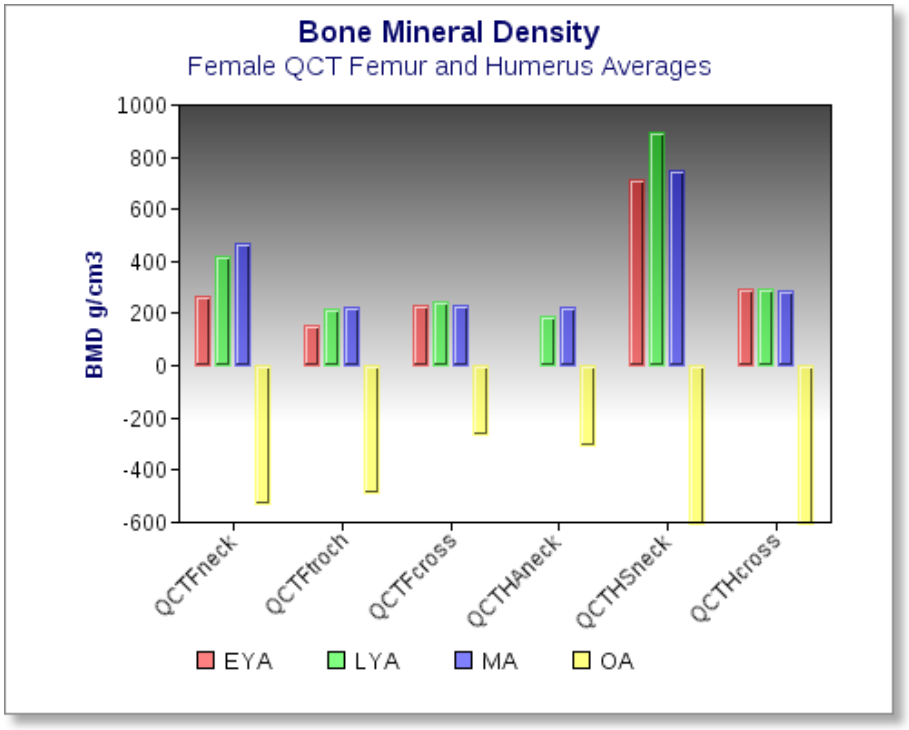

Figure 16: Female QCT average bone mineral density (BMD) for femoral and humeral slices: age to volumatic BMD (g/cm^3) comparison. QCT_{HSnack} and QCT_{Hcross} both far exceed the -600 limit on this graph. (QCT_{HSnack}: -4972.03 and QCT_{Hcross}: -5138.26). EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

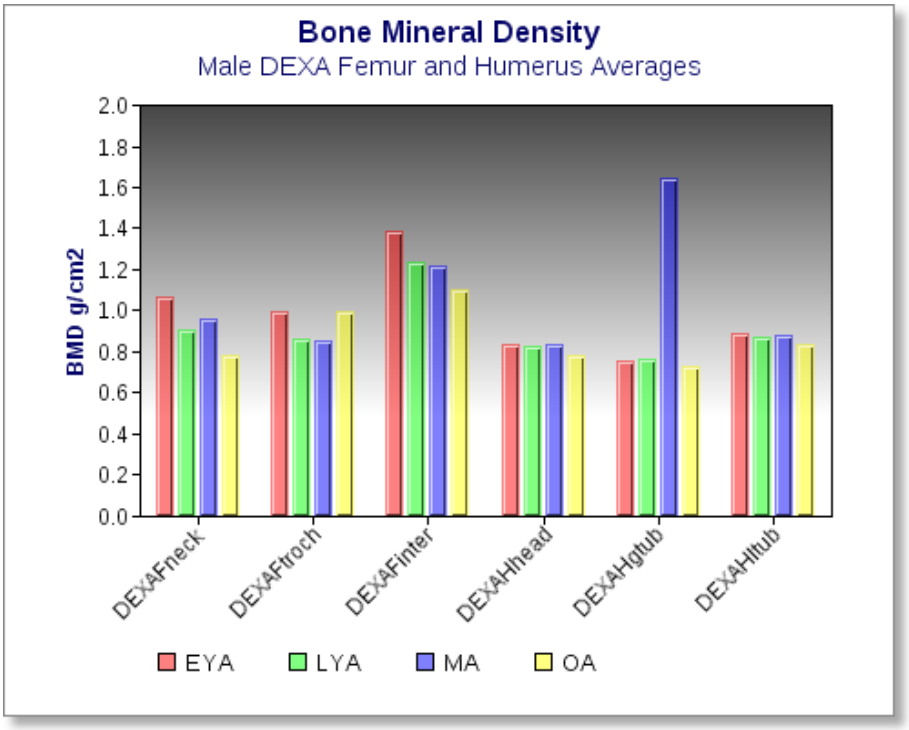

Figure 17: Male DEXA average bone mineral density (BMD) for femoral and humeral slices: age to areal BMD (g/cm^2) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

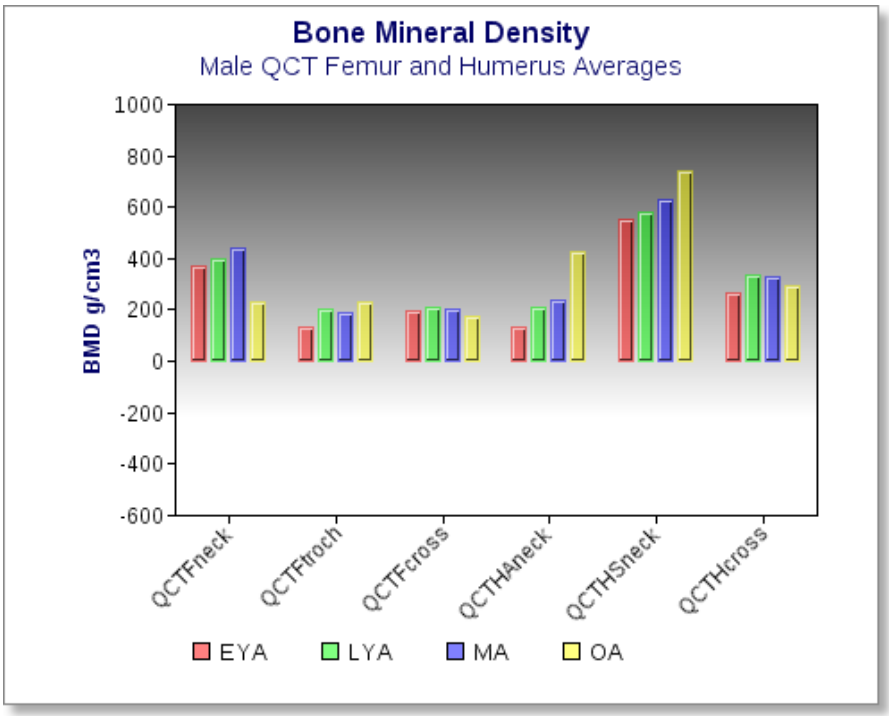

Figure 18: Male QCT average bone mineral density (BMD) for femoral and humeral slices: age to volumatic BMD (g/cm^3) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.3 Trabecular Bone Volume (BV/TV) (fig 19 and 20)

Trabecular bone volume is the amount of total bone volume within the trabecular cavity and is expressed in cm^3.

5.3.1 Femur:

Female trabecular bone volume (BV/TV): Female trabecular bone volume increases from early young adult (EYA) to late young adult (LYA), decrease from LYA to middle adult (MA) and increase again from MA to old adult (OA) for all femoral slices (QCT_{Fneck}, QCT_{Ftroch}, and QCT_{Fcross}).

Male trabecular bone volume (BV/TV): Male BT/TV for QCT_{Fneck} decreases from early young adult (EYA) to late young adult (LYA) and increases from LYA to old adult (OA). QCT_{Ftroch} and QCT_{Fcross} have a similar pattern as the females in that they both increase from EYA to LYA and decrease from LYA to middle adult (MA). However, QCT_{Ftroch} has a much larger decrease from MA to OA than QCT_{Fcross}'s MA to OA decrease.

Femoral BV/TV statistical analyses: There are no statistical differences in femoral QCT trabecular bone volume between males and females and between age categories (table 8). Male only QCT and female only QCT were not analyzed statistically for BV/TV because one or more age brackets had only one individual when subdivided by sex.

5.3.2 Humerus:

Female trabecular bone volume (BV/TV): Female BV/TV for QCT_{HAneck} indicates a decrease in volume from late young adult (LYA) to old adult (OA). Female early young adult (EYA) QCT_{HAneck} data was not available due to scan complications. QCT_{HSneck} EYA to LYA plateau then decrease to middle adult (MA), subsequently increasing to OA. QCT_{Hcross} presents an increase from EYA to LYA and a decrease from LYA to OA.

Male trabecular bone volume (BV/TV): Male trabecular volume QCT_{HAneck} decreases from early young adult (EYA) to old adult (OA). Male QCT_{HSneck} is similar to female QCT_{HSneck} in that they both decrease from late young adult (LYA) to middle adult (MA), and increase from MA to OA. However, male

QCT_{HSneck} increases from EYA to LYA. QCT_{Hcross} EYA to LYA decreases with a slight increase from LYA to MA followed by a decrease from MA to OA.

Humeral BV/TV statistical analyses: As with the femur, there are no statistical differences in femoral QCT trabecular bone volume between males and females and between age categories (table 8). Male only QCT and female only QCT were not analyzed statistically for BV/TV because one or more age brackets had only one individual when subdivided by sex.

5.3.3 Summary:

BV/TV analysis provides no statistically significant differences, however, the following can be determined. Both males and females show an increase in femoral neck (QCT_{Fneck}) and humeral surgical neck (QCT_{HSneck}) trabecular volume between middle adult to old adult. Male and female humeral anatomical neck (QCT_{HAneck}) and QCT_{Hcross} decrease from middle adult to old adult. QCT_{Hcross} have similar male and female trabecular volumes (male 0.539 cm^3; female 0.530 cm^3). Additionally, male and female humeral surgical neck (QCT_{HSneck}) BV/TV decrease from late young adult to middle adult and increase from middle adult to old adult. Male QCT_{Ftroch} (femoral trochanters) OA volume is lower than any other slice.

Table 8: Trabecular bone volume (BV/TV) sex and age statistical data.

	Sex-related BV/TV			**Age-related BV/TV**		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	-0,946	37	0,351	0,570	3	0,638
QCT_{Ftroch}	1,849	37	0,072	0,993	3	0,407
QCT_{Fcross}	-0,885	38	0,382	2,042	3	0,125
QCT_{HAneck}	-0,855	34	0,398	0,787	3	0,510
QCT_{HSneck}	-0,083	34	0,935	1,910	3	0,148
QCT_{Hcross}	-0,915	34	0,367	1,798	3	0,167

* Mann-Whitney U test z value

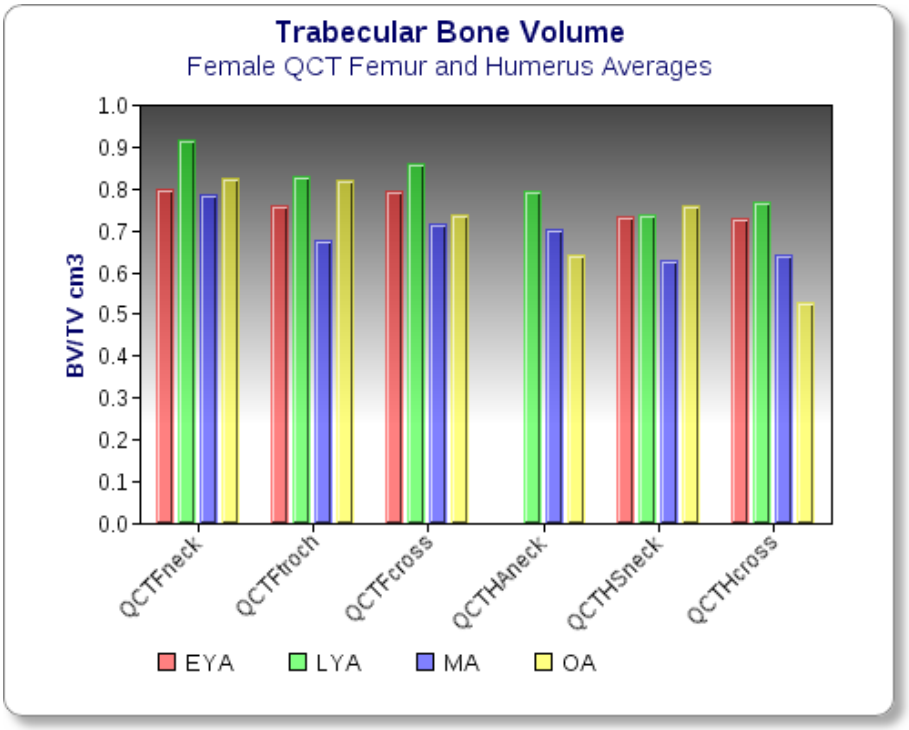

Figure 19: Female QCT average trabecular bone volume (BV/TV) for femoral and humeral slices: age to BV/TV (cm^3) comparison. Note: Female EYA (pink) QCT_{HAneck} data was not available due to scan complications. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

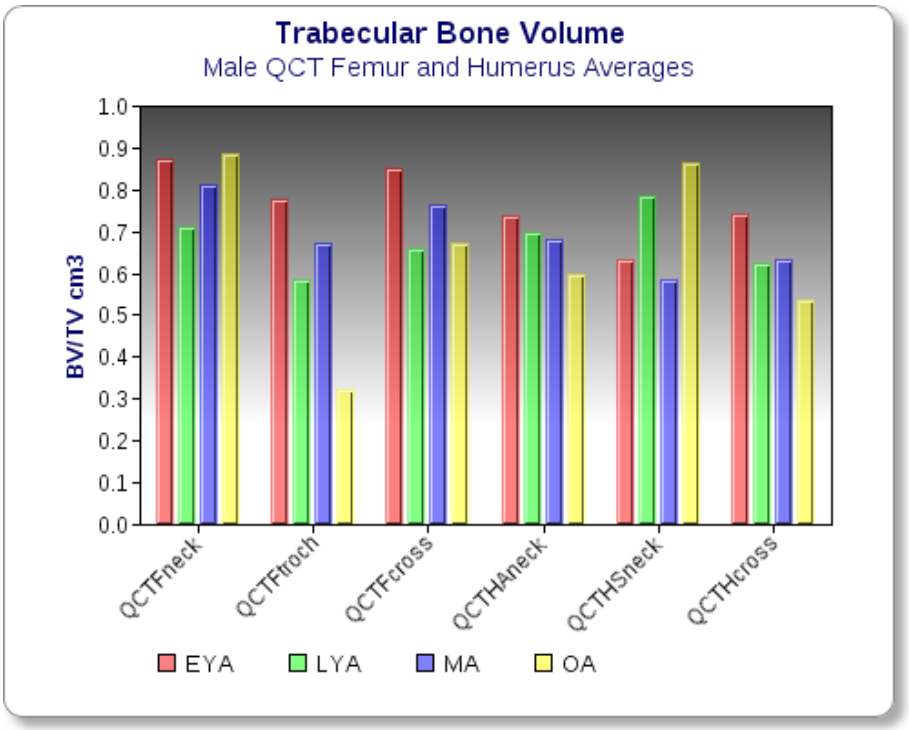

Figure R20: Male QCT average trabecular bone volume (BV/TV) for femoral and humeral slices: age to BV/TV (cm^3) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.4 Trabecular Thickness (TbTh) (fig 21 and 22)

Trabecular thickness is expressed as cm^3.

5.4.1 Femur:

Female trabecular thickness (TbTh): Female femoral trabecular thickness increases from early young adult (EYA) to late young adult (LYA) and decreases from LYA to middle adult (MA) for all three femoral slices ($QCT_{Fneck,}$ QCT_{Ftroch} and $QCT_{Fcross)}$. MA to old adult (OA) thickness decreases in QCT_{Fneck}, increases in QCT_{Ftroch}, and plateaus in $QCT_{Fcross.}$

Male trabecular thickness (TbTh): For males, all three femoral slices ($QCT_{Fneck,}$ QCT_{Ftroch} and $QCT_{Fcross)}$ indicate an increase in trabecular thickness from middle adult (MA) to old adult (OA). However, QCT_{Fneck} early young adult (EYA) to late young adult (LYA) decreases and then plateaus from LYA to MA. EYA to LYA in QCT_{Ftroch} increases and then decreases from LYA to MA. QCT_{Fcross} shows a decrease from EYA to LYA and a slight increase from LYA to MA.

Femoral Tb/Th statistical analyses: There are no statistical differences in femoral QCT trabecular bone thickness between males and females and between age categories (table 9). Male only QCT and female only QCT were not analyzed statistically for TbTh because one or more age brackets had only one individual when subdivided by sex.

5.4.2 Humerus:

Female trabecular thickness (TbTh): Female humeral thickness for QCT_{HAneck} indicates a plateau from late young adult (LYA) to middle adult (MA) with a decrease to old adult (OA). Female early young adult (EYA) QCT_{HAneck} data was not available due to scan complications. QCT_{HSneck} shows an increase from EYA to OA while QCT_{Hcross} increases from EYA to LYA and decreases from LYA to OA.

Male trabecular thickness (TbTh): Male thickness for QCT_{HAneck} suggests a slight decrease from early young adult (EYA) to late young adult (LYA) and an increase from LYA to old adult (OA). The QCT_{HAneck} pattern is similar for QCT_{HSneck} except that QCT_{HSneck} presents a much larger increase from LYA to

OA. QCT_{Hcross} decreases from EYA to LYA, with a plateau to middle adult (MA) and then decreases again to OA.

Humeral Tb/Th statistical analyses: There are no statistical differences in humeral QCT trabecular bone thickness between males and females and between age categories (table 9). Male only QCT and female only QCT were not analyzed statistically for TbTh because one or more age brackets had only one individual when subdivided by sex.

5.4.3 Summary:

Though no statistically significant differences seen with trabecular thickness, the following was observed. Male and female QCT_{Ftroch} exhibit the same pattern of early young adult increase to late young adult, decrease to middle adult, and increase old adult. Overall trabecular thickness is slightly higher in female humeri (0.487 cm^3) than in female femora (0.440 cm^3). Male QCT_{HSneck} for all ages have a higher trabecular thickness (EYA: 0.662 cm^3; LYA: 0.598 cm^3; MA: 1.056 cm^3; OA: 2.309 cm^3) than all other male femoral and humeral slices. QCT_{Hcross} has a similar thickness for males and females (0.316 cm^3 and 0.301 cm^3 respectively).

Table 9: Trabecular thickness (TbTh) sex and age statistical data.

	Sex-related TbTh			**Age-related TbTh**		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	-1,264	37	0,214	0,007	3	0,762
QCT_{Ftroch}	-0,120	37	0,905	1,336	3	0,278
QCT_{Fcross}	0,214	38	0,832	2,043	3	0,104
QCT_{HAneck}	-0,808	34	0,419*	0,225	3	0,878
QCT_{HSneck}	0,215	34	0,831	1,325	3	0,283
QCT_{Hcross}	-1,614	34	0,107*	0,256	3	0,857

* Mann-Whitney U test z value

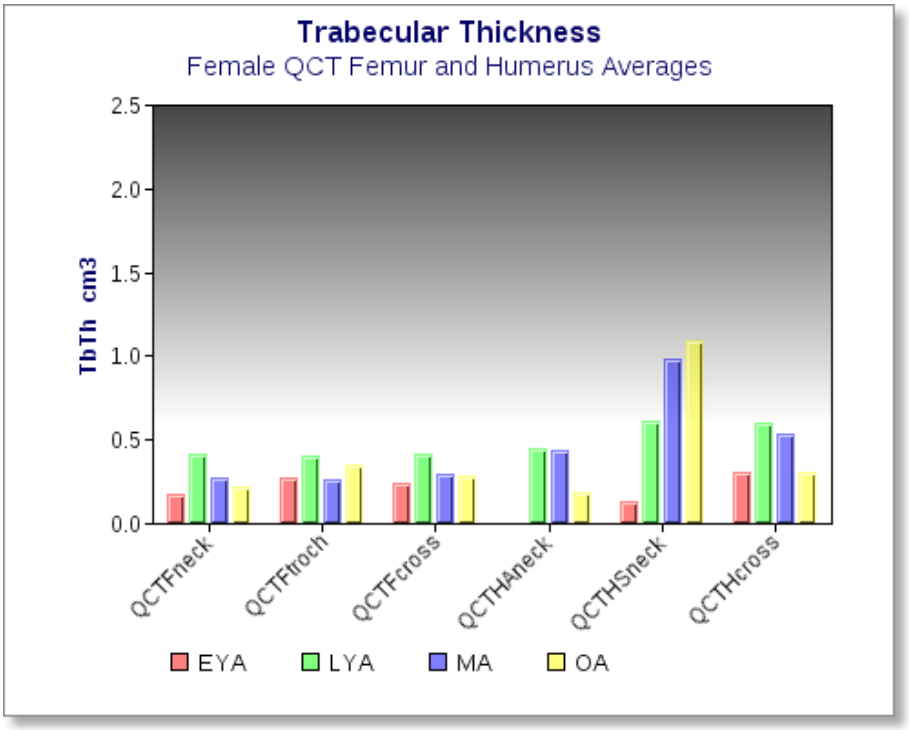

Figure 21: Female QCT average trabecular thickness (TbTh) for femoral and humeral slices: age to TbTh (cm^3) comparison. Note: Female EYA (pink) QCT_{HAneck} data was not available due to scan complications. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

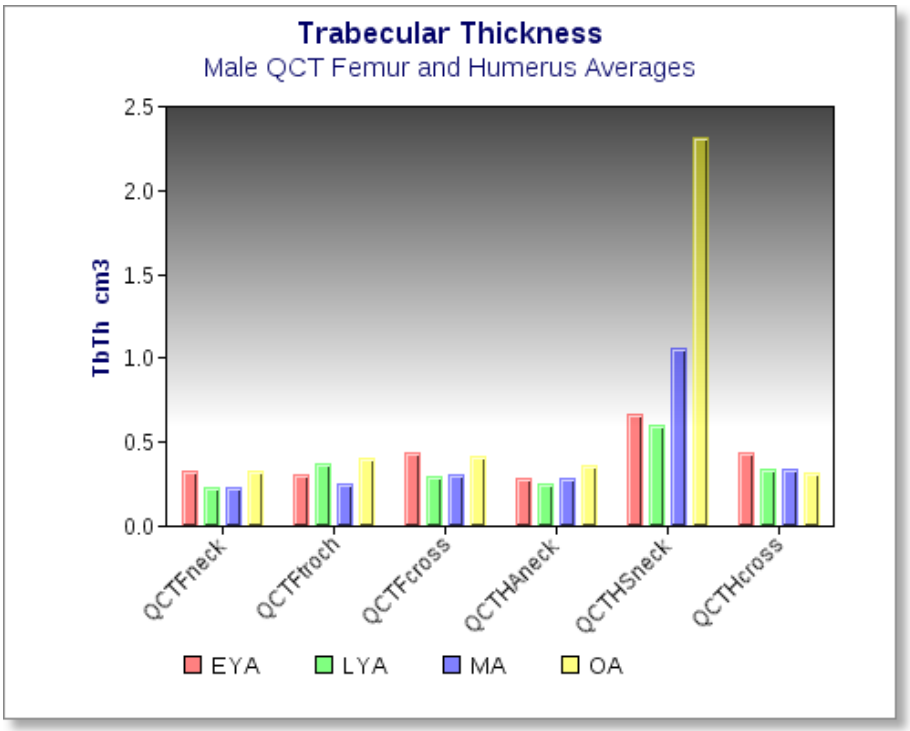

Figure 22: Male QCT average trabecular thickness (TbTh) for femoral and humeral slices: age to TbTh (cm^3) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.5 Trabecular Spacing (TbSp) (fig 23 and 24)

Trabecular spacing is the average space between trabeculae and is expressed in cm^3.

5.5.1 Femur:

Female trabecular spacing (TbSp): Female femoral average trabecular spacing for QCT_{Fneck} increases from early young adult (EYA) to middle adult (MA) and decreases from MA to old adult (OA). QCT_{Ftroch} female trabecular spacing increases from EYA to late young adult (LYA), decreasing from LYA to MA and increasing from MA to OA. QCT_{Fcross} increases from EYA to LYA and decreases from LYA to OA; additionally this slice has almost double the trabecular space than the other femoral slices which may be due to slice volume.

Male trabecular spacing (TbSp): Male QCT_{Fneck} femoral Tb/Sp decreases from early young adult (EYA) to late young adult (LYA) and increases from LYA to old adult (OA). QCT_{Ftroch} indicates a decrease in spacing from EYA to LYA and an increase of space from LYA to OA. EYA to LYA spacing decreases in slice QCT_{Fcross}, plateaus from LYA to middle adult (MA), and increases from MA to OA.

Femoral TbSp statistical analyses: Male QCT_{Fneck} has a statistically significant increase of trabecular spacing over females ($z = -2.068$; $p = 0.039$). There is no trabecular spacing sex-related statistical differences in QCT_{Ftroch} and QCT_{Fcross}. Nor are there significant differences between age categories for all three femoral QCT slices (table 10). Male only QCT and female only QCT were not analyzed statistically for TbSp because one or more age brackets had only one individual when subdivided by sex.

5.5.2 Humerus:

Female trabecular spacing (TbSp): Female $QCT_{HAneck,}$ QCT_{HSneck} and QCT_{Hcross} all show decreased average trabecular spacing from late young adult (LYA) to old adult (OA). QCT_{HSneck} and QCT_{Hcross} have an increase in space for early young adult (EYA) to LYA. Female EYA QCT_{HAneck} data was not available due to scan complications.

Male trabecular spacing (TbSp): Male QCT_{HSneck} and QCT_{Hcross} average TbSp patterns are the same with a decrease from early young adult (EYA) to late young adult (LYA) and an increase from LYA to old adult (OA). QCT_{HSneck} plateaus from EYA to LYA with a slight decrease from LYA to middle adult (MA) and a large decrease from MA to OA.

Humeral Tb/Th statistical analyses: There are no statistical differences in humeral QCT trabecular spacing between males and females and between age categories (table 10). Male only QCT and female only QCT were not analyzed statistically for TbSp because one or more age brackets had only one individual when subdivided by sex.

5.5.3 Summary:

Male average trabecular spacing is statistically higher than females for slice QCT_{Fneck}. All female femoral slices show an increase in average TbSp from early young adult to late young adult while males have a decrease in trabecular space. Additionally, all male femoral slices show an increase in space from middle adult to old adult. Both male and female QCT_{HSneck} old adult spacing is low (male: 0.1 cm^3; female: 0.8 cm^3). Late young adult females exhibit similar humeral spacing for all slices (QCT_{HAneck}: 1.829 cm^3, QCT_{HSneck}: 1.815 cm^3 and QCT_{Hcross}: 1.859 cm^3) as well as late young adult males for QCT_{HAneck} (1.384 cm^3) and QCT_{Hcross} (1.385 cm^3). Female middle adult humeral spacing for QCT_{HAneck} (1.371 cm^3) and QCT_{Hcross} (1.371 cm^3) are the same.

Table 10: Trabecular spacing (TbSp) sex and age statistical data.

	Sex-related TbSp			**Age-related TbSp**		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	-2,068	37	**0,039***	0,827	3	0,488
QCT_{Ftroch}	0,996	37	0,326	1,140	3	0,347
QCT_{Fcross}	0,711	38	0,481	0,798	3	0,503
QCT_{HAneck}	0,116	34	0,908	0,241	3	0,867
QCT_{HSneck}	-1,180	34	0,272	2,352	3	0,091
QCT_{Hcross}	0,276	34	0,784	0,399	3	0,755

* Mann-Whitney U test z value

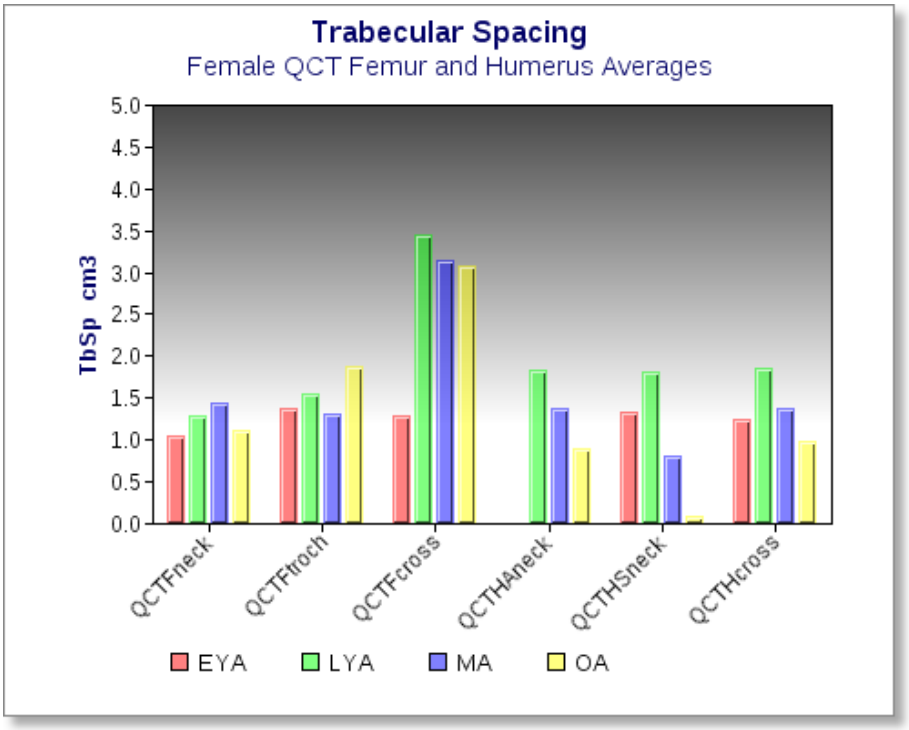

Figure 23: Female QCT average trabecular spacing (TbSp) for femoral and humeral slices: age to TbSp (cm^3) comparison. Note: Female EYA (pink) QCT_{HAneck} data was not available due to scan complications. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

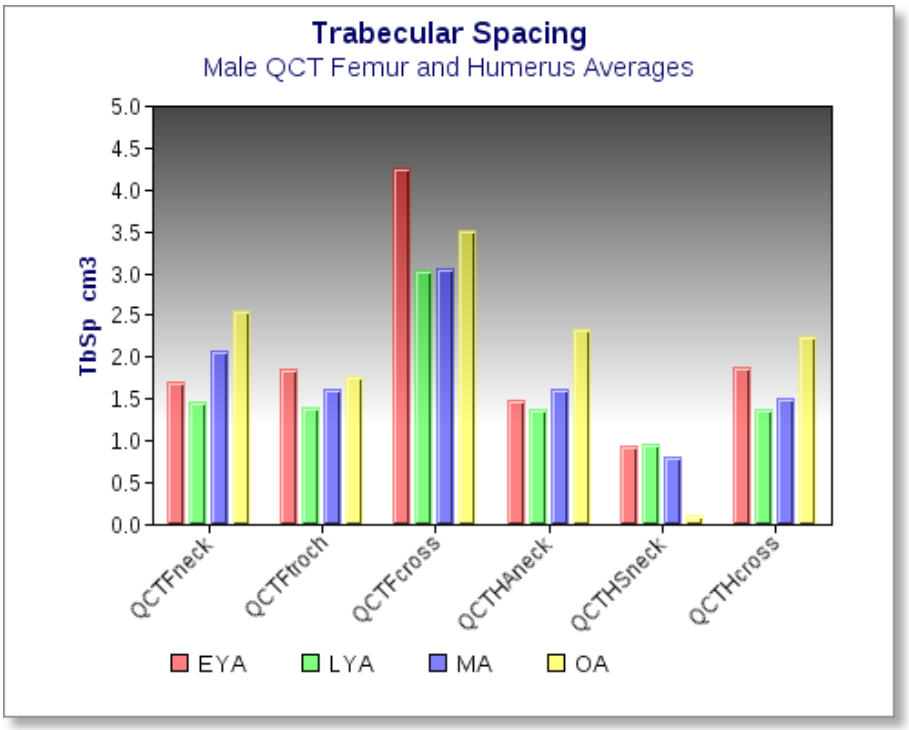

Figure 24: Male QCT average trabecular spacing (TbSp) for femoral and humeral slices: age to TbSp (cm^3)**.** EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.6 Connectivity (Conn) (fig 25 and 26)

Connectivity is the approximate number of trabeculae present.

5.6.1 Femur:

Female connectivity (Conn): Female QCT_{Fneck} and QCT_{Ftroch} both exhibit a decrease in the number of trabeculae (connectivity) from early young adult (EYA) to late young adult (LYA) then plateau to middle adult (MA) with an increase to old adult (OA). Female QCT_{Fcross} trabeculae numbers largely decrease from EYA to LYA, with a smaller decrease to MA and a slight increase to OA.

Male connectivity (Conn): Male femoral connectivity decreases from middle adult (MA) to old adult (OA) in all slices. Trabeculae numbers plateau from early young adult (EYA) to late young adult (LYA) for QCT_{Fneck} and then increase to MA. Both QCT_{Ftroch} and QCT_{Fcross} indicate a decrease from EYA to LYA with an increase to MA.

Femoral Conn statistical analyses: Males have statistically more connectivity than females in QCT_{Fneck} ($t = 2.446$; $df = 37$; $p = 0.19$) and QCT_{Fcross} ($t = 2.030$; $df = 38$; $p = 0.049$). No significant difference was seen between the sexes for QCT_{Ftroch}, nor are there significant differences between age categories for all three femoral QCT slices (table 11). Male only QCT and female only QCT were not analyzed statistically for Conn because one or more age brackets had only one individual when subdivided by sex.

5.6.2 Humerus:

Female connectivity (Conn): Female humeral connectivity changes are the same for early young adult (EYA) to middle adult (MA) in slices QCT_{HSneck} and QCT_{Hcross} with a decrease from EYA to late young adult (LYA), and a plateau from LYA to MA. MA to old adult (OA) for QCT_{HSneck} increases, while it decreases in QCT_{Hcross}. Additionally, QCT_{HAneck} trabeculae numbers slightly increase from LYA to OA. Female EYA QCT_{HAneck} data was not available due to scan complications.

Male connectivity (Conn): Male trabeculae counts plateaus from early young adult (EYA) to late young adult (LYA) and decrease from LYA to old adult (OA) for both QCT_{HAneck} and $QCT_{HSneck.}$ For male QCT_{Hcross}, trabeculae

numbers decrease from EYA to LYA, plateau from LYA to middle adult (MA), and decrease from MA to OA.

Humeral Conn statistical analyses: Males have a statistically higher number of trabeculae than females for the humerus; QCT_{HAneck} ($t = 2.932$; $df = 34$; $p = 0.006$), QCT_{HSneck} ($z = 3.869$; $p = 0.001$) and QCT_{Hcross} ($t = 3.526$; $df = 34$; $p = 0.001$). There are no significant differences between age categories for all three humeral QCT slices (table 11). Male only QCT and female only QCT were not analyzed statistically for Conn because one or more age brackets had only one individual when subdivided by sex.

5.6.Summary:

Two main patterns are present for connectivity. First, males and females have a steady increase in the number of trabeculae from QCT_{Fneck} to QCT_{Ftroch} to QCT_{Fcross}. This is expected because each slice has a larger volume and as the volume increases the number of trabeculae should increase as well. The only exception for this is with old adult males, where there is a decrease in trabeculae for QCT_{Ftroch}. Second, the overall lower number of trabeculae for all ages in slice QCT_{HSneck} for both male and female is also due to the total volume of the slice. As with the $QCT_{Ftroch,}$ old adult male QCT_{HSneck} has fewer trabeculae. Males statistically have more trabeculae in their humeri, femoral necks, and QCT_{Fcross} than females. All three humeral slices for females from late young adult to middle adult differ by no more than five trabeculae [QCT_{HAneck} (LYA: 77.214, MA: 81.722), QCT_{HSneck} (LYA: 30.75, MA: 27.611), and QCT_{Hcross} (LYA: 69.143, MA: 67.083)] as well as female femoral slice QCT_{Ftroch} (LYA: 113.071, MA: 113.812).

Table 11: Connectivity (Conn) sex and age statistical data.

	Sex-related Conn			Age-related Conn		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	2,446	37	**0,019**	0,479	3	0,699
QCT_{Ftroch}	1,245	37	0,221	2,063	3	0,123
QCT_{Fcross}	2,030	38	**0,049**	1,502	3	0,231
QCT_{HAneck}	2,931	34	**0,006**	0,832	3	0,486
QCT_{HSneck}	-3,470	34	**0,001***	2,074	3	0,123
QCT_{Hcross}	3,526	34	**0,001**	1,208	3	0,323

* Mann-Whitney U test z value

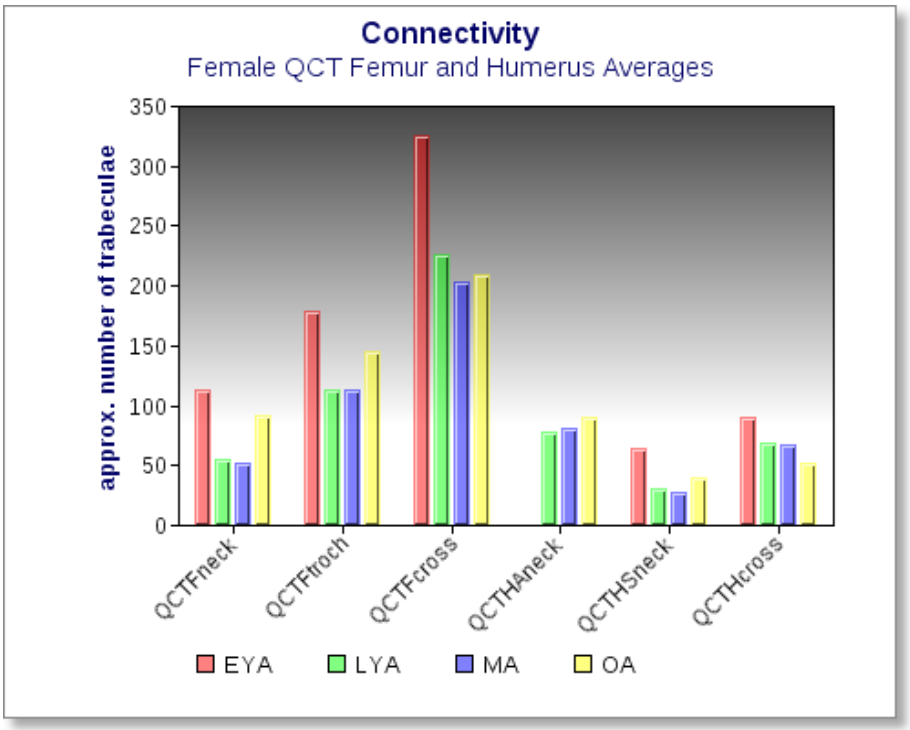

Figure 25: Female QCT average connectivity (Conn) for femoral and humeral slices: age to approximate number of trabeculae comparison. Note: Female EYA QCT_{HAneck} data was not available due to scan complications. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

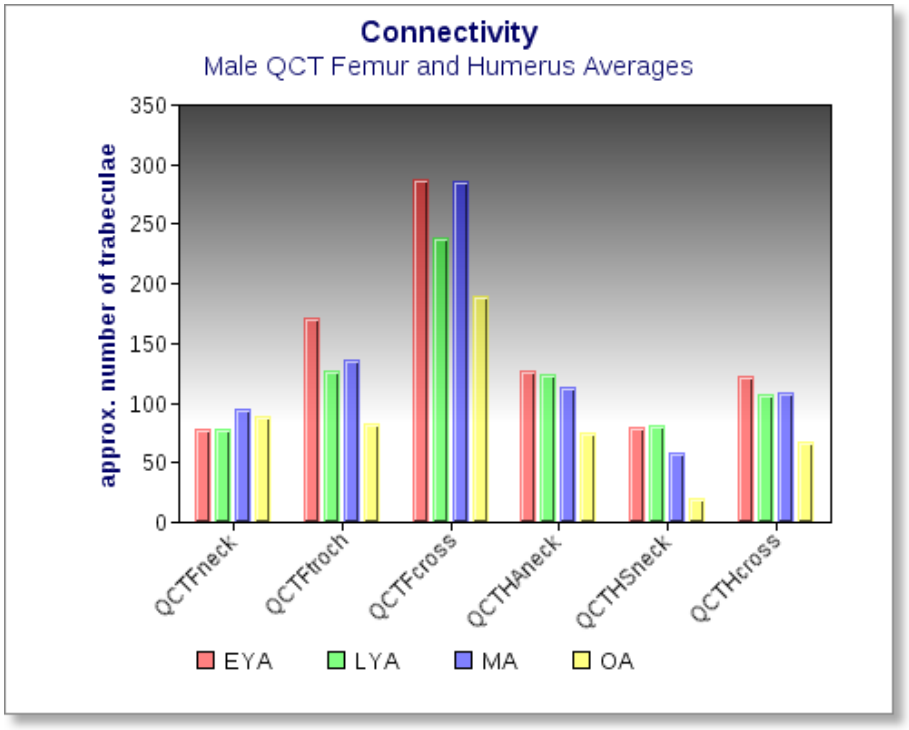

Figure 26: Male QCT average connectivity (Conn) for femoral and humeral slices: age to approximate number of trabeculae comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.7 Connectivity Density (Conn.D) (fig 27 and 28)

Connectivity density is the number of trabeculae per unit volume and is expressed in cm^3.

5.7.1 Femur:

Female connectivity density (Conn.D): Female femoral connectivity density for QCT_{Fneck} and QCT_{Ftroch} have a similar pattern indicating a decrease from early young adult (EYA) to middle adult (MA) and an increase from MA to old adult (OA). Female QCT_{Fcross} indicates a decrease from EYA to OA.

Male connectivity density (Conn.D): All three male femoral slices QCT_{Fneck}, QCT_{Ftroch} and QCT_{Fcross} have the same pattern with a decrease from early young adult (EYA) to late young adult (LYA), increase from LYA to middle adult (MA) and decrease from MA to old adult (OA).

Femoral Conn.D statistical analyses: There are no statistical differences in femoral QCT connectivity density between males and females and between age

categories (table 12). Male only QCT and female only QCT were not analyzed statistically for Conn.D because one or more age brackets had only one individual when subdivided by sex.

5.7.2 Humerus:

Female connectivity density (Conn.D): Female QCT_{HAneck} indicates an increase from late young adult (LYA) to old adult (OA). Female EYA QCT_{HAneck} data was not available due to scan complications. Both QCT_{HSneck} and QCT_{Hcross} show a decrease of connectivity density for females early young adult (EYA) to middle adult (MA) with QCT_{HSneck} increasing in OA while decreasing in $QCT_{Hcross.}$

Male connectivity density (Conn.D): All male humeral slices show a decrease in connectivity density from early young adult (EYA) to old adult (OA). However, EYA to late young adult (LYA) for QCT_{HAneck} and QCT_{Hcross} only slightly decreases by 1 cm^3 and 3 cm^3 respectively.

Humeral Conn.D statistical analyses: Like the femur, there are no statistical differences in humeral QCT connectivity density between males and females and between age categories (table 12). Male only QCT and female only QCT were not analyzed statistically for Conn.D because one or more age brackets had only one individual when subdivided by sex.

5.7.3 Summary:

Male femoral slices present the same pattern of change between age groups for trabecular connectivity density: decrease, increase, decrease. Male old adult has lower connectivity density for both the femur and humerus while females have both lower and higher reading for both the femur and humerus respectively. There are no significant differences in connectivity density (table 12).

Table 12: Connectivity density (Conn.D) sex and age statistical data.

	Sex-related Conn.D			Age-related Conn.D		
	t value	df	sig p value (two-tailed)	f value	df	sig p value (two-tailed)
QCT_{Fneck}	0,185	37	0,854	0,662	3	0,581
QCT_{Ftroch}	-0,661	37	0,513	0,794	3	0,505
QCT_{Fcross}	-0,441	38	0,661	0,963	3	0,421
QCT_{HAneck}	1,309	34	0,199	0,426	3	0,736
QCT_{HSneck}	-1,790	34	0,073*	2,519	3	0,076
QCT_{Hcross}	1,409	34	0,168	0,544	3	0,656

* Mann-Whitney U test z value

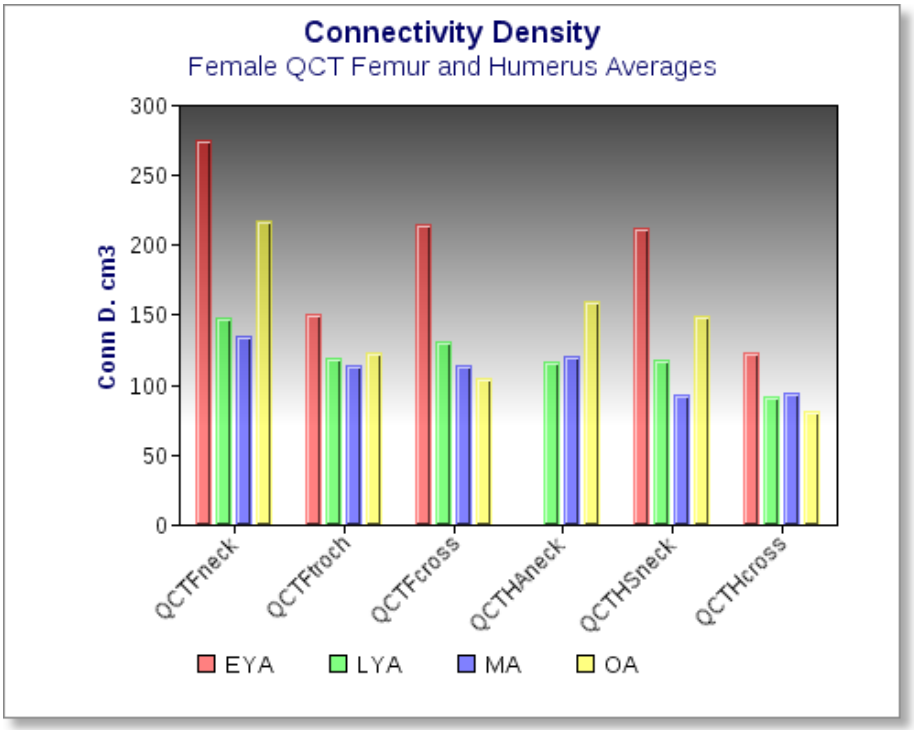

Figure 27: Female QCT average connectivity density (ConnD) for femoral and humeral slices: age to ConnD (cm^3) comparison. Note: Female EYA QCT_{HAneck} data was not available due to scan complications. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

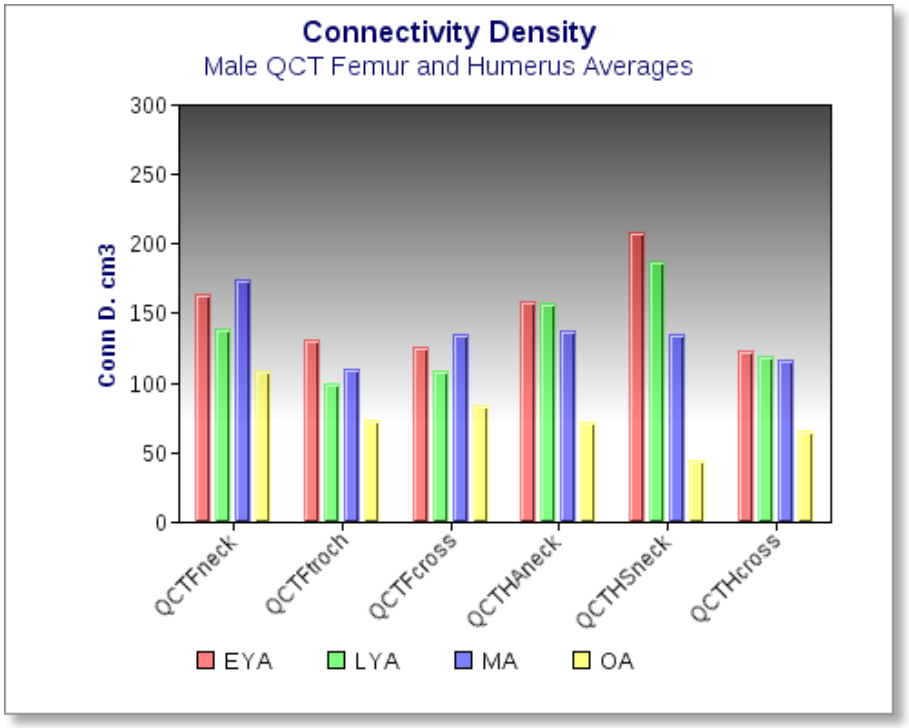

Figure 28: Male QCT average connectivity density (ConnD) for femoral and humeral slices: age to ConnD (cm^3) comparison. EYA = early young adult (18 – 25 years). LYA = late young adult (26 – 34 years). MA = middle adult (35 – 49 years). OA = old adult (50+ years).

5.8 Cross Comparison

5.8.1 DEXA and QCT BMC

Bone mineral concentration (BMC) per slice comparisons for DEXA and QCT indicate that DEXA BMC data is an average of 98.5 % lower than QCT BMC ($DEXA_{Fneck}$ and QCT_{Fneck}: 99.33%. $DEXA_{Ftroch}$ and QCT_{Ftroch}: 99.03%. $DEXA_{Finter}$ and QCT_{Fcross}: 97.54%. $DEXA_{Hhead}$ and QCT_{HAneck}: 95.86%. $DEXA_{Hgtub}$ and QCT_{HSneck}: 99.67%. $DEXA_{Hltub}$ and QCT_{Hcross}: 99.06%). The consistency of the differentiation between DEXA and QCT for specific slices indicates that while the data are different they are comparable. It may be possible to write future equations to calibrate the difference between DEXA and QCT. Future research is needed to determine if this is possible for the Middenbeemster assemblage and if it can be applied to other agriculture populations.

Further break down by element indicates that DEXA humeral BMC is 62% lower than femoral BMC (female only at 66%, male only at 58%). QCT humeral BMC is 15% lower than femoral BMC (female only at 19%, male only at

11%). This suggests that QCT BMC readings are 47% lower than DEXA BMC readings (per element for the whole sample, females only and males only) indicating that if either of these methods were used for BMC analysis, a calibration curve of 47% would be included so that a comparison between cortical QCT BMC and total DEXA BMC could be made. However, this result may be distorted for the following resasons. Further breakdown by age, excluding male and female old adult for DEXA and QCT (because of a highly negative female old adult $_{v}$BMD QCT reading, humerus and femur, most likely due to a small sample size n = 1), indicates a 39% to 43% difference between methods for BMC. Further analysis of each age group (males and females combined) indicated that again there is a difference of 40 to 41% for each method for early young adult, late young adult and middle adult. The old adult individuals have a humeral BMC 65% lower than the femur for DEXA while QCT indicated old adult femura had a 4% lower BMC than old adult humeri. This result is interesting because it seems that the inclusion of the old adult female QCT data creates a consistent percentage change between data collection methods and thus an indication of a possible calibration curve. It must be noted that a calibration curve is not within the scope of this thesis. This result was an unexpected conclusion and should be researched to its fullest extent, with a larger sample size, at a later date.

5.8.2 DEXA and QCT BMD

Bone mineral density (BMD) comparison differences between DEXA and QCT did not produce any coherent results, most likely due to differences in area/volume of each slice, the trabecular bone and cortical bone combination for DEXA, and only cortical for QCT, and again, because of the female old adult with highly negative $_{v}$BMD. This negative $_{v}$BMD is not normal. All BMD readings should be positive values. There are a few possibilities as to why this female reading was so low. As stated above, a small sample size was used (n = 1). No soft tissue substitute was used for QCT scans, and the scanning program calculates for tissue density. Air has a value of -1000 and thus if the cortical tissue is either highly porous or BMC readings were not taken correctly, then the reading could be affected.

5.8.3 Whole Bone Correlations

Whole bone correlations are based on age-related whole bone patters (all femoral slices and all humeral slices) of decrease or increase for each bone per sex. While statistically there are is no significance to these observations unless stated above for individual slices, they provide a better understanding of the bone changes seen in the Middenbeemster population.

Female femora (all slices): Female femora indicate that an increase of BMC, trabecular volume, trabecular thickness, and spacing are observed until reaching approximate peak bone mass (late young adult ages 26 – 34). But there is a decrease in the number of trabeculae and their connectivity density which may be due to the presence of thicker, denser, and more spaced-out trabeculae. After reaching peak bone mass, the female proximal femur decreases in both cortical and trabecular bone mineral concentration. Subsequently, trabeculae start to thin, causing lower trabecular volume and less connectivity density and therefore causing lower $_{a}$BMD due to bone loss. As females reached old age (50+ years), an increase in aBMD coupled with continued loss in whole bone mineral concentration (DEXA BMC) and cortical vBMD (QCT). The bone gain indicated by aBMD was somewhat unexpected but not unrealistic with such a small sample size (n = 1). This increase in old age female femoral BMD can be assumed to represent individual variation rather than population variation within the Middenbeemster collection.

Female humeri (all slices): Until estimated peak bone mass (late young adult 26 to 34 years) no female humeral pattern can be seen. A decrease in aBMD, trabecular volume, and trabecular spacing are present from late young adult to middle adult (35 to 49 years). Continuing into old age, trabecular spacing increases in conjunction with lower $_{a}$BMD and $_{v}$BMD. As with the female femur, bone loss can be seen after peak bone mass is surpassed.

Male femora (all slices): Male femora have lower $_{a}$BMD, less trabecular volume, with less connectivity density prior to reaching approximate peak bone mass age (late young adult, 26 to 34 years). Males subsequently have an increase in proximal femoral trabecular volume in association with an increase in both the

number of trabeculae and their connectivity density into middle adulthood (35 to 49 years). As expected with old age (50+ years), male cortical femoral bone mineral concentration (QCT) and trabecular connectivity density decrease. However, in this sample, this is also associated with an increase in trabecular thickness and spacing, suggesting that while increased femoral bone loss is associated with increased age, cortical and trabecular bone degrade at different rates.

Male humeri (all slices): Until reaching approximate peak bone mass (late young adult age 26 to 34), male humeri increase in bone mineral concentration (QCT) and vBMD. The number of trabeculae and connectivity density continue to decrease through old adulthood (50+ years). No other clear whole bone male humeral pattens are present. However, it can be assessed that humeral bone loss is seen as males age.

Load bearing and non-load bearing: This assessment of pattening, while simple, indicates that bone loss is present within the Middenbeemster assemblage post-approximate peak bone mass. However, load bearing and non-load bearing elements exhibit different data type factors to indicate bone loss. Load-bearing bones consistently exhibit changes in trabecular bone volume until age 35 to 49 (middle adult) followed by lower bone mineral concentration into old age (50+ years), while non-load bearing bones exhibit changes in bone mineral density from early young adult (18 to 25 years) to old adult (50+ years). In general, as seen above, femora have a larger number of patterning data types that can be used to evaluate bone loss, while the humerus does not. Additionally, for both elements males are more likely to have changes in the number of trabeculae and therefore connectivity. Females have more changes when it comes to $_{a}$BMD and $_{v}$BMD for both the femur and humerus. Further research into load bearing and non-load bearing definitive data type changes is needed because it has the potential to help evaluate bone loss when only a few data types are obtainable.

5.9 Result summary

Bone mineral concentration (BMC) and bone mineral density (BMD) for the femur and humerus can be measured with DEXA and QCT but BMD

comparisons between elements and machines are unreliable. BMD decreases with age and can be more clearly seen with DEXA for both male and female, femur and humerus scans, than with QCT suggesting that areal $_{a}$BMD is more reliable than volumatic $_{v}$BMD (table 13). However, it should be noted that female femoral $_{a}$BMD increase from middle adult to old adult. It could be assessed that this is a individual variant and would not affect the overall Middenbeemster population. BMD, while standard, is individualized based on the diet, genetics, race, smoke, etc (see chapter 2) and thus this result is part of the normal population variation for this populations. Modern population studies also indicate that increase in BMD can sometimes be seen as individuals age (Mays *et al.* 1998). With that, and the lack of osteoporotic fractures within this sample, marked shifts in bone loss cannot be clearly defined. That is, there is now way to define what bone loss rates are in the Middenbeemster assemblage nor when there would have a high enough risk of fracture to determine the average age when osteoporosis would be present.

Table 13: Female and male bone mineral concentration (BMC) and bone mineral density (BMD) averages: all ages, femur and humerus, DEXA and QCT.

Age	Sex	DEXA Femural *BMC [(g)]*	QCT Femoral HU [(g)]	DEXA Femoral $_{a}$*BMD [g/cm^2]*	QCT Femoral $_{v}$BMD [g/cm3]	DEXA Huneral *BMC [(g)]*	QCT Humeral HU [(g)]	DEXA Humeral $_{a}$*BMD [g/cm^2]*	QCT Humeral $_{v}$BMD [g/cm3]
EYA	F	33,49	709,07	1,126	218,437	12,89	597,14	0,769	335,815
LYA	F	35,51	1054,13	1,167	295,354	12,76	813,17	0,773	457,930
MA	F	31,03	975,34	1,000	305,524	11,86	776,76	0,698	420,251
OA	F	43,43	981,19	1,141	-414,682	11,01	823,22	0,665	-3471,487
	Average Female	35,86	929,93	1,109	101,158	12,13	752,57	0,726	-564,373
EYA	M	44,82	911,16	1,212	234,464	17,47	677,75	0,836	319,414
LYA	M	44,32	983,60	1,073	272,797	18,17	817,39	0,827	376,829
MA	M	44,04	1085,12	1,136	278,053	18,29	869,03	0,840	398,216
OA	M	42,43	606,99	1,032	214,401	19,09	838,46	0,780	487,756
	Average Male	43,90	896,72	1,113	249,929	18,26	800,66	0,821	395,554

In most cases BMC is higher in the femur and humerus in males than in females for DEXA scans. The only exception is with one QCT slice (QCT_{Fcross}) having similar BMC old adult readings for males and females. Bone mineral

concentration (BMC) comparison between DEXA and QCT is possible indicating that a calibration curve may be possible to equate and that slice locations on each element are consistent when using different machines. Even if the readings on QCT are higher, this can be taken into account and, again, possibly calibrated for in the future. A larger sample size is needed to confirm this BMC machine correlation.

Both males and females have an increase in trabecular bone volume (BV/TV) in the femoral neck and humeral surgical neck from middle adult to old adult QCT data, with a corresponding decrease in BV/TV in their humeral anatomical necks and humeral cross section (QCT_{Hcross}). Females have a slightly higher trabecular thickness in their humeri than their femora with QCT_{HSneck} thickness relatively higher for males and females than all other slices. In general, males have more spacing between trabeculae in their femoral necks than females. Spacing plateaus for all late young adult (26 to 36 years) humeral reading, male and females, suggesting a plateau in trabecular change during this age which may be because of reaching peak bone mass. Males significantly have more connectivity (number of trabeculae) in their humeri then females with females having similar numbers of trabeculae for all three humeral slices from late young adult to middle adult. Again this may be due to reaching peak bone mass. Femoral trabeculae counts in the male femoral neck are also significantly higher than females. The additions of QCT trabecular assessment indicate that there is a stronger correlations between the different data types for the femur than for the humerus. A complex interaction between BMC/BMD and trabecular volume, thickness/spacing, number of trabeculae, and connectivity density can be seen. Thus the additions of QCT trabecular assessment helps support bone loss within this population, in that it provides additional details about internal bone structural changes.

6 Discussion

6.1 QCT and DEXA: does this really work?

Bone loss assessment is possible with archaeological dry bone. Even though it has been suggested that current medical software may have problems when scanning without a wet soft tissue substitute (Roberts and Manchester 2007). No standard frailty curve could be determined for the Middenbeemster sample which may be in part due to scanning differences between machines. Agarwal and Grynpas (2009) indicated that BMD obtained with DEXA on archaeological populations have reported contradicting results on bone loss with some samples exhibiting patterns similar to modern populations such as an increased bone loss in postmenopausal women while others exhibit loss earlier in life, similar patterns between males and females and/or minimal age-related loss. This is true for the Middenbeemster population in that an increase in female $_{a}$BMD is seen between middle adult and old adult while QCT $_{v}$BMD indicates an average decrease in bone density as both sexes age in both the humerus and femur. The data suggests that while females are more likely to exhibit changes in trabecular volume in load bearing bones (the femur). While this change has been relatively consistent, it causes additional problems in assessing aBMD because DEXA readings are size dependent, they are better correlated with height and weight (table 14). Thus suggesting that vBMD may be a better suited for assessing female bone loss. Female non-load bearing bones are more correlated to decreased in bone mineral density readings (aBMD and vBMD) suggesting that size dependency for this element is irrelevant.

Male data suggests that both load bearing and non-load bearing elements are affected more by differences in connectivity (the number of trabeculae) and trabecular density. This imples that makes have more dence trabelaue then women which could be associated with increased strength and robuststitisy. This futher can be correlated with increased activity within the population. Saers (2012) cross-sectional geometry research on sexual dimorphism suggests that males load bearing bones were stronger and more robust than females in Middenbeemster

however female data presented high variability indicated that women preformed a wide range of tasks that included activities involving heavy loading. This assessment can futher be confirmed with the increase in aBMD seem within Additionally humeri were strongest in men than females. Therefore suggesting that Middenbeemster males were stronger than females. However, cortical loss between age groups was not clearly distinguished suggesting that the Midddenbeemster inhabitants were more active. Saers (2012) analyses help support bone loss differences in elemental analysis between the sexes but does correlate with age changes pertaining to bone loss. Further research is needed to examine cross sectional geometry and bone loss in this assemblage.

Table14: Technical aspect comparison of DEXA and QCT as presented by Engelke et al (2008).

	DEXA	QCT
Image Type	2D	3D
Materials that can be scanned	Bone and soft tissue	Bone, soft tissue, ceramics, egyptain mummies (etc)
Bone type	Bone: cortical and trabecular combined	Cortical and trabecular combined, cortical only, trabecular only
Measurements	BMC and BMD	BMC, BMD, Trabecular architecture, connectivity, connectivity density, cross sectional geometry
Measurement readings	Area density (g/cm^2), length (mm and cm)	Volumatic density (g/cm3 and mg/cm^3), length (mm and cm), numerical
Calibration	Phantom at beginning or the day (all machines are different)	Phantom with each scan (all machines the same)
T-score validity	Valid	Invalid
Image distortion	Magnification of proximally 7%	none
Reading dependency	Bone size dependent (better correlated with height and weight)	Bone size independent
Resolution	Fuzzy, can distinguish cortical and trabecular in general	↓ slice thickness = ↑ geometric resolution, ability to distinguish individual aspects of scanned object
Field of view		Whole body
Region of interest	Predesignated, rectangular box	Slice of any thickness in any image plane
Positioning	Correct positioning prior to scan	Ability to manually rotate image into desired position after scan.
Soft tissue	Needed, archaeological material must be scanned with a soft tissue substitute	Not needed but a soft tissue substitute can be used with archaeological material

This studies data indicates that the addition of QCT examination of trabecular bone helped in determining bone loss. Yet, problems do arise when using different methods to assess bone loss. Table 14 summarizes the technical aspects of QCT and DEXA methodologies. It is not the authors intent to examine all differentiating aspects between each method used for that is not the in the scope of this analysis. However, the following discussion sections focus on some of the major issues encounter during bone loss assessment and how they can affect bone loss assessment of archaeological material.

6.1.1 The problem with bone mineral density

Valid bone mineral density reading were obtained with both DEXA and QCT indicated the presence of bone loss within the Middenbeemster assemblage. As reviewed in chapter 1, bone mineral density (BMD) can be measured in volume or area. BMD does not represent true density (Hassager and Christiansen 1995), rather it is an expression of the amount of bone mineral content (BMC) for a given area or region of interest (ROI).

$$\text{Area BMD} \qquad \text{BMD}(g/cm^2) = \frac{\text{BMC (g)}}{\text{Area (cm)}}$$

$$\text{Volume BMD} \qquad \text{BMD}(g/cm^3) = \frac{\text{BMC (g)}}{\text{Area (cm)} * \text{Area thickness (cm)}}$$

However when a bone is scanned, a software program calculates BMD by averaging the pixel density of the ROI. In actuality, BMD (Heaney 2005, 1013) is:

$$\text{BMD (per unit area)} = \frac{\text{bone mineral concentration behind a bone shadow}}{\text{shadow area}}$$

Over the past few years, BMD has been heavily re-examined due to its misuse as an indicator of increased bone loss. While it does show bone loss, BMD does not indicate bone mass and trabecular architecture which are the basis of strength. Additionally, bone density varies per skeletal element (Damilakis *et al.* 2007). For archaeological material, this is a big issues. the main problem seems to lie with T-score and Z-score analysis. T-scores are derived when the BMD measurement is subtracted from a 'young healthy reference' mean BMD and divided by the standard deviation. The problem is that the 'young healthy reference' BMD is from a modern population. In some ways, this method can works if the reference is from the same ethnic background (different ethnicities

exhibit different rates of bone loss) as the archaeological population in question; however, this is never for certain and should be done with caution. A Z-score is derived when the BMD is subtracted from mean age and a matched reference then divided by the standard deviation. This may be more efficient for archaeological purposes, however the question then is: how large does the matched reference sample have to be when assessing archaeological material? This question cannot be answered at this time. It can be suggested, based on the data derived from this study, that while BMD does indicate bone loss, bone mineral concentration may be is a more reliable data source especially when examined in conjunction with trabecular architectural aspects. With that, until future research indicates that bone mineral density is completely unreliable, its utilization will remain relevant in bone loss studies.

6.1.2 DEXA

DEXA bone mineral density provided clear patters in bone loss for females in both the femur and humerus between age groups. Female humeri $_{a}$BMD steadily decaeased with increase age and femoral $_{a}$BMD indicated an eventual increase into old age. Male $_{a}$BMD was more complex however on average, femora and humeri both indicated lower $_{a}$BMD in old age. Female bone mineral concentration was also than males for both the femur and humerus.

Extensive utilization of DEXA has made this methodology the gold standard for assessing bone loss in present and past populations (Adams 2008; Carey and Delaney 2010). It should be noted that when scanning with DEXA three vital pieces of information need to be inputted for analysis to be preformed. The technician must indicated ethnicity, height and weight of the individual. In archaeological population this information can only be estimated.

However a few problems persist when using DEXA to assess bone loss. DEXA is designed for one thing and one thing only; to assess bone loss through BMC and BMD through 2-dimentional x-rays. The problem is that scans are expensive and archaeological funding is limited. So why then do we still rely on DEXA? It most likely lies with a relatively high accuracy (three to eight percent) and precision (one to five percent) rate (Adams 2008). But on order to obtain

accuracy and precision, a custom made jig is recommended to hold the element in place while scanning. For this study, bags of dry rice were used as a soft tissue substitute (another downfall of DEXA, there has to be a soft tissue substitute or the machine will not register that it is scanning a bone) which provided easy manipulation of the element into the "correct" scan position. However, this position was estimated and subjected to placement error that can change density readings because of misalignment. The second issues is that DEXA cannot distinguish between ante-mortem and post-mortem bone changes (Roberts and Wakely 1992) because a clear image cannot be produced. DEXA scans are relatively blurry and only a general differentiation between cortical bone and trabecular bone can be determined. Figures 29 and 30 provide a visual comparison between QCT and DEXA image types. Additionally, DEXA image quality can affect BMC. It has been magnification caused though scanning does not significantly affect bone mineral density it does alter bone mineral concentration (Adams 2008). Thus, bone mineral concentration analysis should be cautioned.

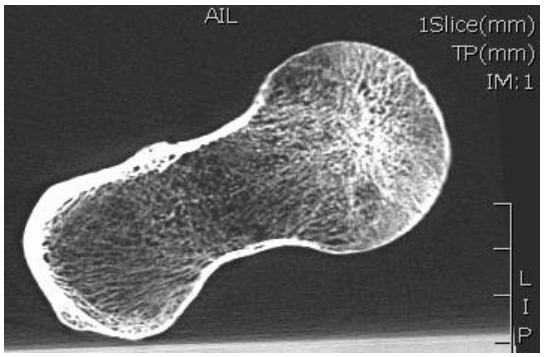

Figure 29: QCT slice QCT_{Fcross} from MA female (MB11S045V0055). Notice clarity of trabeculae when compared to figure 30, DEXA femoral scan from the same individual.

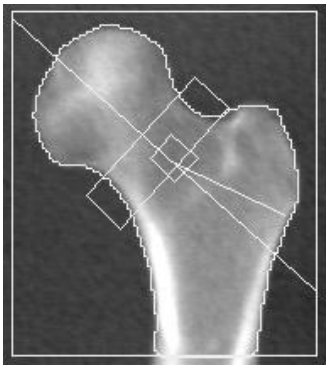
Figure 30: DEXA femoral scan of MA female (MB11S045V0055). Notice how trabeculae cannot be clearly assessed.

6.1.3 QCT

QCT trabecular assessment proved additional viable information about bone loss even though $_{v}$BMD provided conflicting results. Female $_{v}$BMD produced a hightly negative old adult value. As stated earlier, a four point average was used to obtain average cortical BMC and $_{v}$BMD because original exploration of test individuals produced highly negative results; air equals HU -1000. It is

believed that this was due to no soft tissue substitute added while scanning. Again it must be stated that bone should have a positive reading (van Rijn and van Kuijk 2008). Theoretically, however, if this method was not used the standard HU -1000 could have been accounted for and thus still produced usable data that was consistently off by -1000. However, this may have caused more problems, for with increased bone loss, there is increased trabecular cavity spacing creating an even higher negative reading for old adult individuals or those that exhibited trabecular damage. Measurements produced by current methods need to be calibrated to account for the absence of soft tissue. Because of this, Gonzales-Reimers *et al*. (2007) suggested that QCT is an inaccurate method to use on archaeological material.

Another factor is that the elements were manually rotated with help by Ruff (2002) diagrams to place each element in anatomical position creating interpersonal error for each rotation that was undertaken. Manual rotation was used so that more than one element could be scanned at a time. A custom made jig is suggested to hold each element in anatomical position while scanning so that later excessive rotations are not needed, but this will increase the number of scans needed to complete ones study. "Artifacts" are different density lines that are produced when the scanning beam interacts with the object that is scanned. They were not taken into account during this study and thus potentially could alter all QCT readings that were obtained in this study. It should be noted that the more elements scanned together, the more an increase of "artifacts" becomes present within the field of view. Complete elimination of artifacts is not possible but scanning each bone individually will dramatically decrease the presence of "artifacts."

The ability to produce accurate 3-dimentiaoal scans that clearly show trabecular architecture and be examined for multiple aspects (table 14) of bone physiology suggests that this method is highly underused when assessing archaeological material. The overall problem with QCT is that it is relatively inexcusable because in clinical setting it is relied upon for all types of analysis, not just bone physiology (van Rijn and van Kuijn 2008).

6.2 The humerus? A good indicator nonetheless?

DEXA indicated that humeral BMC was 62 % lower than the femural BMC when all individual were grouped together, (66% lower for only females and 58% lower for only males). QCT humeral BMC were 15%, 19% and 11% lower respectively. First, as stated in the results section, there is a consistent 47% difference between DEXA and QCT BMC scores. BMD DEXA indicate that the humerus is 31% lower than the femur for all individuals, (34% lower for females and 26% lower for males). QCT BMD readings did not produce consistent results with BMD with the humerus being lower by 148% for all individual, 648% lower for females but males exhibited an increase in humeral BMD (18% higher than the femur). A study byDoetsch *et al.* (2002) suggests that humeral BMD may be a better indicator to age related bone loss than the hip. Their study of 80 Danish women, age 30 – 81 years, scanned with DEXA, indicates that there is a correlation between the proximal humerus and proximal femur. In their study, the humerus presents a 15% lower BMD, correlated with increase in age, most likely because it is non-load bearing and smaller in size. Additionally, this seems to support the theory regarding calcium migration and load bearing bone as discussed by Parfitt (2003). For this thesis, DEXA humeral BMD is approximately double the 15% difference. This may be due to the use of rice as a soft tissue substitute, as well taphonomic and burial environment factors that affect skeletal remains. Further research on dry bone scans is needed to confirm that the humerus is constantly lower than the femur and at what average percentage it is.

Rose *et al.* (1982) examined medical records, radiographs and autopsy reports collected within a ten year period (1965 – 1974) pertaining to the entire population of Rochester, Minnesota USA for humeral fractures. During this period 586 humeral fractures (proximal, distal, and/or diaphysal) were reported, affecting 564 individuals, 338 women and 226 men. Of these, 249 initial and 25 recurrent fractures pertained to the proximal humerus (n = 274). There is an increased incidence of individuals over age 30 with most fractures occurring in

the proximal humerus (10% distal, 14% diaphyseal, and 76% proximal). More woman were affected proximal humeral fractures then men that were associated to moderate trauma, falls from a standing position, that did not affect the greater tuberosity. The lack of greater tuberosity damage is thought to be due to increased risk of rotator cuff disease with age, which would cause a tear in the elderly and fracture in young individuals. Kelsey *et al.* (1992) conducted a study of 9704 American women over age sixty-five and determined that there is an increased risk of proximal humeral fractures associated with lower BMD, poor nutrition, and decreased activity. The Middenbeemster female $_{a}$BMD indicates a steady decrease associated with increased age however decreased activity is yet to be determined.

In short, the humerus can be a good indicator for bone loss but a standardized methodology needs to be established as well as rate differences between the humerus and femur for archaeological material. This study has brought to light a correlation between methods for humeral and femoral BMC. This study also begins the exploration of the use of the humerus as a valid indicator for bone loss in past populations. The ability to include an additional element in evaluation bone loss wan the femur is not present or highly degraded is desired. As research progresses, it is hoped that this element will become a standard addition in the examination of bone loss.

6.3 Defining bone loss in the archaeological record

The determination of the presence or absence of osteopenia within the archaeological assemblage is a challenge. The misuse of the term osteopenia within the literature has called for an expansion of the simplistic definition so that clearly defined parameters can be established in order to standardize the term for osteoarchaeolgical research. T-scores and z-scores are not acceptable assessment scores in osteoarchaeology because they are compared with modern populations and should thus be excluded. Current software programs cause concern because of automatic soft tissue calibration when scans are used to determine BMD and should be used with caution. BMD calculations themselves cause a contradictory assessment because the equation shows that an increase in bone size will cause a

decrease in strength while in reality larger bones are normally stronger (Heaney 2005).

Throughout this thesis the term "bone loss" has been used because of these factors. So then the question becomes how does one then define osteopenia so that it can be used for bone loss assessment in past populations? It is not completely possible to come up with a strict definition because the variables are too complex. I suggest that a clearer picture may be formed if the parameters of osteopenia are defined. The following observations can be made: 1) T-scores and z-scores cannot be used for archaeological material. 2) Current programs can be utilized however caution must be taken and calibrations need to be made. 3) Comparisons between groups (example: hunter gatherer and agricultural) are improbable at best. There are too many variables that can not be accounted for. 4) BMD alone is the standard for modern populations but needs to be taken with caution for archaeological material in that soft tissue calibration is needed. 5) Using multiple machines and multiple measurements is best to see a clearer picture of the past. Reliance on individual procedures, while economical, can provide inaccurate results. In the end, I strongly argue that the term "osteopenia" cannot be used for bone loss in past populations because there are too many factors that are built upon assumptions when dealing with skeletal material. Therefore, at this point, no sold shift in bone loss can be seen prior to onset of osteoporosis and the onset of osteoporosis is still yet to be seen.

6.4 Post-Mortem Modification

Problems arise with any methodology, especially when using archaeological material. A main problem is destruction of bone microstructure due to biological and chemical diagenesis. Jackes et at. (2001) examined archaeological bone from Portugal dating back to the Mesolithic for microstructural changes and compared them to experiments conducted on modern bone in order to view the rates of microbial destruction. Their research indicated that bacteria can cause bone mineral alterations such as a conversion of hydroxyapatite to octocalcium phosphate (hydroxyapatite precursor). This form of diagenesis can have massive implications for BMD readings because bacterial

change is not universally distributed within the burial environment. The rate of taphonomic change needs to be taken into account yet a solid method of determining diagenesis rates for archaeological material has not been developed and may not be possible due to the extreme variability of burial environments. These problems are unavoidable however, knowledge of how these factors influence bone loss assessment in past populations is necessary for a clearer understanding of bone loss in the past.

QCT scans indicated soil infiltration in some of the elements including some elements that had no external damage. These elements were kept within the analysis sample because of an already small samples size. Additionally, hypothetically, if only DEXA scans were used then soil infiltration would not be clearly seen and thus not accounted for. Preservation, taphonomic processes such as weathering and degradation can create an imbalance in the sample material preventing proper analysis.

7 Future Research

"The past is but the beginning of a beginning, and all that is or has been is but the twilight of the dawn."
H.G. WELLS, *The Discovery of the Future*

7.1 QCT and DEXA research

DEXA is the gold standard for studying bone loss is modern populations. However, while it does produce viable data for archaeological material it can only assess bone mineral concentration and bone mineral density in whole, undamaged, the proximal femur, lumbar vertebrae and distal radius. The three key locations examined to assess osteoporotic fracture risk. QCT has many promising aspects to enhance our understanding of bone loss in past populations. Not only does QCT provide bone mineral concentration and bone mineral density data, it can accurately produce 3-dimentional replications of any object that is scanned that can be saved digitally for later use. One of its greatest advantages is the ability to analyses broken or damaged skeletal elements. As with any method, as scanning technology becomes better acquainted for archaeological material and cheaper to use, it will be utilized more frequently. Further advancements in QCT methodology and analytical software are needed to increase precision and accuracy in using this technology to assess bone loss in skeletal assemblages..

7.2 Addition of the humerus in archaeological study

Proximal femur, lumbar vertebrae and distal radius DEXA BMD measurements are well known locations for determining age-related bone loss rates in archaeological populations. Current research of the proximal humeral fracture rates in modern populations have provided us with yet another location that can be looked at for osteopenia and osteoporosis. It has been shown that BMD in this region is 15% lower than that of the femur in modern populations (Doetsch et al. 2002) and in this thesis has shown the rate to be ~30% (DEXA) indicating that when preservation of the proximal femur is poor, the humerus can be used to determine a valid BMD score for skeletal material of the past. One of the biggest problems in archaeology is preservation within the burial environment. In some cases, only a few elements from an individual will survive and be

testable. Thus, the humerus can also be examined in conjunction with the femur, lumbar vertebra and distal radius.

7.3 Extension of the Middenbeemster assemblage

The determination of frailty rates after the age of 50 are not possible for archaeological material in normal circumstances unless we have exact age at death and radiographic and/or histological analysis is preformed. However, death records for the Middenbeemster assemblage provide exact ages at death for some of the individuals in the collection. Thus, it is possible, with further research to determine the different frailty curves for all ages, but especially the elderly. Correlations can then be made, depending on the outcome, and associated to osteoarchaeological age brackets. In essence, we will be able to provide an estimated rate of bone loss for Dutch 19th century agricultural communities. Literary review indicates that only one other archaeological assemblages has been analyzed that has death records, the material from Wharram Percy (Mays 1996).

As more research is done on the Middenbeemster collection, a secondary review of the data presented in this thesis can be undertaken. As discussed throughout, the study of age-related bone loss is a complex interacting web of variables that all play into the primary, secondary and tertiary influences of bone loss. Future analysis of these results with increased background information will provide a better understanding of bone loss within rural agricultural populations.

7.4 Standardized methodology and specialized software for archaeological bone loss assessment with modern machines.

It is naive to assume that one standard methodology with specialized software could be produced to assess bone loss in archaeological populations that is user friendly, inexpensive, easily accessible and accurate. At this time it is not a realistic goal. I do propose that different methodologies be assessed to determine if calibration curves could be developed so that similar data obtained through different methods could be compared to some extent. As seen in this study, an unexpected result obtained from the data analysis was a 47% lower reading in QCT than DEXA. Future research into calibration curves and/or methodology

standardization for the assessment of bone loss in archaeological assemblages, has a lot of potential to increase our understanding of bone loss rates in the past.

8 Conclusion

Bone loss studies are extremely complex. The interaction of the multiple factors that cause bone loss is not fully understand. The term osteopenia should not be considered for archaeological material because its definition is based on modern population T-scores and true frailty curves are not possible without a large sample size. Therefore frailty rates for the Middenbeemster could not be determined. This study has shown that yes, bone loss can be assessed within archaeological dry bone material with both DEXA and QCT. The Middenbeemster population did exhibit age related bone loss in both males and females that could be seen in both the femur and the humerus. The inclusion of trabecular architecture analysis with QCT supports these findings by providing further detail as to how bone loss occurs between males and females in all age groups. This study also suggests that the humerus is a good alternative element to use to evaluate bone loss when femora are not present or highly damaged. However, a consistency in bone loss was not seen indicating that further research into the Middenbeemster population is needed to explain these finding .

Abstract

Age-related bone loss has been receiving a lot of attention in recent years in an attempt to assess and treat conditions that are affected by bone loss such as osteopenia. Current bone loss influences are reviewed as well as an overview of bone loss assessment in archaeological material. This research evaluated age-related bone loss in a19th Century Dutch osteoarchaeological population to determine frailty rates through Quantitaitive Computed Tomography (QCT) and Dual- Energy X-ray Absoorptiometry (DEXA) of loading (femur) and non-loading (humerus) elements. An examination of areal bone mineral density (aBMD) and volumatic (vBMD) were explored in correlation to trabecular architecture. It was determined bone loss was present within in the population however, the onset of osteoporotic fracture risk was unable to be determined. Male loading and non-loading bones exhibit consistent changes in trabecular connectivity and connectivity volume while females are more likely affected by an increase in trabecular bone volume. Humeral BMC data between DEXA and QCT indicated that a possible calibration curve can be determined suggesting that the humerus is a good indicator of age-related bone loss. Subsequently, the term "osteopenia" was determined to be invalid for this assessment and the term "bone loss" is thus suggested to be used.

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Appendix A: Material Catalogue

Identification	Left Humerus	Left Femur	Right Humerus	Right Femur	Age	Sex	Stature (cm)	Body Mass (kg)	Pathology
S045 V0055	X	X			MA	F	158.70 ± 3.55	71	Vertebral lipping, schmoral's nodes
S053 V0290		X	X		MA	F	160.51 ± 3.55	60	vertebral lipping, possible sinisitis,
S059 V0133	X	X			MA	M	171.71 ± 2.99	74	
S092 V0124	X			X	LYA	M	177.69 ± 2.99	72	vertebral lipping, schmorl's nodes
S149 V0280	X	X			EYA	PF	155.78 ± 3.55	53	Slight Scoliosis, vertebral schmorls nodes
S187 V0311	X	X			LYA	F	168.21 ± 3.55	49	
S198 V0601		X	X		LYA	F	167.47 ± 3.55	48	
S226 V0282	X	X			MA	M	178.34 ± 2.99	76	
S233 V0304	X	X			MA	M	179.45 ± 2.99	84	
S236 V0335	X	X			EYA	M	170.80 ± 2.99	77	Vertebral schmorl's nodes
S239 V0369	X	X			EYA	M	162.56 ± 2.99	70	
S243 V0381	X	X			MA	F	166.49 ± 3.55	62	
S261 V0422			X	X	OA	M	167.42 ± 2.99	88	Cranial depressions, porosity on R femur on medial condyle, large microporosity on lateral R humeral head
S290 V0472	X	X			EYA	M	168.59 ± 2.99	70	L tibia: bony projection at medial collateial ligament attachemnt, femur not affected

Identification	Left Humerus	Left Femur	Right Humerus	Right Femur	Age	Sex	Stature (cm)	Body Mass (kg)	Pathology
S306 V0561		X	X		LYA	M	172.49 ± 2.99	77	
S310 V0550	X	X			LYA	M	177.79 ± 2.99	81	L femur: allen's fossa, criba anterior next just inferior of head. Criba also on R femor, minor cribrial orbiatalia
S313 V0926	X	X			MA	M	177.07 ± 2.99	67	
S337 V0714	X	X			OA	M	183.54 ± 2.99	85	Dental: periapical granuloma and peridontitis
S356 V0864	X			X	MA	PF	155.37 ± 3.55	65	
S360 V0762	X	X			OA	PF-F	167.04 ± 3.55	62	Osteoarthritis, L femur: lipping and osteophyes of proximal margines of head, head has eburnation. Vertebral lipping, schmoral's nodes and eburnation
S368 V0794	X	X			LYA	M	164.56 ± 2.99	71	Vertebral lipping, osteochondritus
S369 V0886	X	X			LYA	F	155.92 ± 3.55	62	Bony thickness superior of R femoral medial condyle, vertebral schmorls nodes
S370 V0806	X	X			LYA	F	172.16 ± 3.55	48	
S374 V0861	X	X			OA	M	163.39 ± 2.99	72	Enthesopathies: L & R humeri tricepts brachii, L & R femur linea aspera and gluteus maximus, L& R tibia. Osteoarthritis. Vertebral lipping. Minor lipping articular facet of L & R Tibia. Bathrocephaly
S379 V0851	X	X			EYA	M	179.25 ± 2.99	70	T6 sacralization on R side
S388 V0952	X	X			EYA	F	171.21 ± 3.55	69	Dental: osteoarthritis of TMJ, granuloma, carries
S401 V0876	X	X			LYA	F	159.95 ± 3.55	55	Bowing of R tibia and Fibula ~ healed racture or Rickets, Osteoarthritis, vertebral schmoral's nodes

Identification	Left Humerus	Left Femur	Right Humerus	Right Femur	Age	Sex	Stature (cm)	Body Mass (kg)	Pathology
S402 V0907		X	X		MA	M	167.55 ± 2.99	67	L humerus: lateral superior pitting between head and greater tubercle. L femus: small bony growth superior of fovea capitis Cribra femorison R & L Femoral necks. Vertebral lipping and chmorals nodes. Healed rib fractures
S413 V0896		X	X		MA	F	148.95 ± 3.55	51	Extra bone formation on vertebrea
S427 V0938	X	X			LYA	M	169.24 ± 2.99	81	
S428 V0945	X	X			MA	F	132.99 ± 3.55	53	Achondroplasia, Osteoarthritis
S430 V0965	X	X			LYA	F	169.82 ± 3.55	63	Vertebral lipping and schmoral's nodes
S432 V0981	X	X			LYA	M	170.02 ± 2.99	72	Vertebral lipping
S435 V0929	X	X			MA	M	170.54 ± 2.99	74	
S453 V0973	X	X			LYA	F	158.15 ± 3.55	69	
S461 V0990	X	X			EYA	M	179.93 ± 2.99	69	
S466 V1010	X	X			MA	F	160.80 ± 3.55	65	
S467 V1022	X	X			LYA	M	171.97 ± 2.99	79	Vertebral schmoral's nodes
S468 V1009	X	X			MA	F	157.59 ± 3.55	58	R tibia and fibula medial bowed, left tibia midshaft normal. Aka right lower limb pathology, left okay. Vertibral lipping and fusion of L5 and S1
S473 V1003	X	X			LYA	M	187.31 ± 2.99	84	Vertebral lipping and schmorals nodes

Identification	Left Humerus	Left Femur	Right Humerus	Right Femur	Age	Sex	Stature (cm)	Body Mass (kg)	Pathology
S476 V1054	X	X			MA	PF	169.82 ± 3.55	69	L5 inferior depression
S481 V1046		X	X		LYA	F	177.84 ± 3.55	69	
S482 V1048	X	X			MA	M	177.04 ± 2.99	77	
S487 V1096			X	X	LYA	F	169.54 ± 3.55	64	Vertebral schmorl's nodes, sacral lesion
S497 V1059		X		X	LYA	M	173.27 ± 2.99	76	Healed spiral fracture of the L tibia and Fibula. Vertebrea schmoals nodes and lamallar spurring. Cribra orbitalia and porotic hyperostosis
S501 V1097	X	X			EYA	F	152.17 ± 3.55	53	Premature surture closure, cranial pitting
S502 V1062	X	X			EYA	M	179.12 ± 2.99	69	
S505 V1095	X	X			EYA	PM	174.69 ± 2.99	72	
S512 V1005		X	X		MA	F	164.26 ± 3.55	62	
S521 V1150	X	X			MA	PF	169.40 ± 3.55	64	Healed rib (7th?) fracture and R masotid erosion
S527 V1053	X	X			LYA	F	175.87 ± 3.55	64	