pQCT Assessment at the Radius and Tibia: The Effects of Menopause and Breast Cancer Therapy on Trabecular and Cortical Bone

pQCT Assessment at the Radius and Tibia: The Effects of Menopause and Breast Cancer Therapy on Trabecular and Cortical Bone

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Abstract

This thesis focuses on an examination of cortical and trabecular bone density and geometry at the radius and tibia in postmenopausal women, primarily women with history of breast carcinoma, while also assessing musculoskeletal changes in postmenopausal breast cancer patients after treatment with the Aromatase Inhibitor, Anastrozole. The first sub-study is an investigation of the reproducibility of the pQCT measurement parameters at the radius and tibia in healthy pre- and postmenopausal women. Results indicated that the reproducibility was good at the radius and even better at the tibia for all parameters measured. The second study is an appraisal of the level of osteoporosis knowledge in a cohort of postmenopausal women. The participants were assessed via the Facts on Osteoporosis Quiz, a well validated questionnaire, and the data revealed significantly lower test scores among the breast cancer subjects in comparison with healthy postmenopausal women. In the remaining group of studies, pQCT technology was utilized to describe trabecular and cortical bone at the radius and tibia in postmenopausal women and women with a history of breast carcinoma whom had been prescribed Anastrozole. The following measurement sites were significantly lower in the breast cancer subjects: TOT_DEN and TOT_CNT at the 4% radius; CRT_DEN, TOT_CNT, and CRT_CNT at the 20% radius; TOT_DEN at the 4% tibia; and CRT_DEN at the 38% tibia. With respect to time on Anastrozole, TOT_CNT at the 4% radius (r=-0.36); TOT_CNT (r=-0.33), CRT_CNT (r=-0.34) and CRT_DEN (r=-0.44) at the 20% radius; and CRT_DEN (r=-0.39) and CRT_CNT (r=-0.27) at the 38% tibia were significantly negatively correlated with days on Anastrozole. Furthermore, after two years of Anastrozole treatment in a small cohort of breast cancer subjects, there was a significant decrease in CRT_DEN

($p=0.025$) at the 20% diaphyseal radius and also at the 38% diaphyseal tibia ($p=0.051$). Together, the sub-studies that comprise this thesis demonstrate that there are noteworthy deficiencies in osteoporosis knowledge among postmenopausal women, particularly those with a history of breast carcinoma, and yet, these are the same women that have an increased need to understand the preventative and treatment options regarding this disease as they demonstrate reduced bone density at all measurement sites. It also appears that time on Anastrozole primarily affects cortical bone density in these women. In summary, this thesis provides novel details regarding cortical bone in breast cancer subjects and emphasizes the need for a normative database of bone quality parameters at different skeletal sites in order to gain a better understanding of the utility of each skeletal site with regard to fracture risk prediction.

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I am especially thankful to the women who served as participants in the studies that comprise this thesis; their sincere desire to contribute to the scientific field and to provide a better prognosis for future breast cancer patients mirrors my own aspirations.

Above all, I am deeply grateful to Matthew - my husband and best friend, my indispensable critic and devoted fan; and to my darling Evelyn - she brightens my life in countless ways.

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Abbreviations

AAM	Age at Menopause
ALP	Bone Alkaline Phosphatase
aBMD	Areal Bone Mineral Density
BC	Breast Carcinoma
BCS	Breast Cancer Subjects
ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMU	Bone Modeling Unit
BMI	Body Mass Index
CRT_CNT	Cortical Content [mg/mm]
CRT_DEN	Cortical Density [mg/cm^2]
CT	Computed Tomography
CRT_THK	Cortical Thickness
DXA	Dual Energy X-Ray Absorbtiometry
E	Young's Modulus
ENDO_CIR	Endocortical Circumference
ER	Estrogen Receptor
et al.	Latin; and others
FOOQ	Facts on Osteoporosis Quiz
g	Grams
HCS	Healthy Control Subjects
i.e.	Latin; that is
IL-1	Interleukin-1
IL-6	Interleukin-6
kg	kilogram
m	meter
M-CSF	Macrophage Colony Stimulating Factor
n	Sample size
NO	Nitric Oxide
NOC	Number of Children
NTX	Urinary N-telopeptide
PERI_CIR	Periosteal Circumference
PMI	Polar Moment of Inertia
PMR	Polar Moment of Resistance
PQCT	Peripheral Quantitative Computed Tomography
PTH	Parathyroid Hormone
*rms*CV	Root Mean Squared Coefficient of Variation
*rms*SD	Root Mean Squared Standard Deviation
SD	Standard Deviation
SEM	Standard Error of the Mean
SSI	Stress Strain Index
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor- alpha
TOT_A,	Total Bone Area [mm^2]
TOT_CNT	Total Bone Content [mg/mm]
TOT_DEN	Total Bone Density [mg/cm^2]
TRAB_A	Trabecular Area [mm^2]
TRAB_CNT	Trabecular Content [mg/mm]
TRAB_DEN	Trabecular Density [mg/cm^3]
vBMD	Volumetric Bone Mineral Density
vs	versus
WHO	World Health Organization
YSM	Years Since Menopause
μ-CT	Micro-Computed Tomography
μSv	Microsievert

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Chapter 1

RESEARCH DESIGN

1.1 INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue which leads to bone fragility and an increased risk of fracture. The essential basis for the development of osteoporosis is an imbalance in bone remodeling, whereby the bone resorption rate exceeds the bone formation rate. This results in a net loss of bone. Specific ratios of cortical and trabecular bone tissue play an important role in maintaining skeletal integrity. The loss of connectivity within trabecular bone and increased porosity of cortical bone reduces both the quality and the quantity of bone. Conversely, suitable apportionment of these distinct tissue types strengthens bone and enables it to adapt to recurrent strains.

Recent research has revealed that areal bone mineral density alone is not sufficient for predicting fractures in the general population; dual energy x-ray absorptiometry (DXA) can identify the presence of low bone mineral density, but cannot predict which individuals with low bone mineral density will endure a fracture. Bone quality is a concept that has emerged to describe the diverse factors that influence fracture risk which extend beyond areal bone mineral density. Peripheral quantitative computed tomography (pQCT) is a bone imaging technique that measures volumetric bone mineral density and cross-sectional bone geometry at peripheral skeletal sites: the radius and the tibia. This device has definite advantages over DXA, as density can be measured in cortical and trabecular bone compartments separately. pQCT also allows for the study of the spatial distribution of the bone material. For example, in cortical bone this spatial distribution is represented by geometric properties such as the polar moment of inertia, the mean cortical thickness, and the stress-strain index.

Estrogen exerts a multitude of actions on bone tissues and is integral to bone health, with estrogen withdrawal during menopause leading to accelerated bone loss and increased risk of fracture. After menopause, intrinsic production of estrogen from androgens largely occurs in non-ovarian tissues such as fat, muscle, skin, and liver as a result of the activity of the aromatase enzyme complex. There is evidence to suggest that even these low levels of circulating estrogens have protective effects on the skeleton. In bone, where estrogen is required to maintain density, there is indication of increased turnover in patients prescribed Aromatase Inhibitors. Aromatase Inhibitors have begun to play an expanded role in the treatment of patients with estrogen-receptor positive breast carcinoma. At present, assessment of the degree of bone disease in postmenopausal breast cancer patients is often incomplete and may exacerbate and increase the risk of fragility fractures due to inappropriate management and treatment.

Further research into the effects of menopause and breast cancer therapy on bone density and bone quality will improve the ability of health care providers to address the bone health needs of postmenopausal women and, therefore, potentially reduce the risk of secondary skeletal complications in this patient population.

1.2 Experimental Approach

The aim of the present thesis is to accurately describe trabecular and cortical bone density and geometry, muscle and fat area, and osteoporosis knowledge, in healthy postmenopausal women and to compare these parameters to a reference population of breast cancer patients of comparable age. A further aim is to document changes in volumetric bone density and

geometry of a subgroup of women after 12 and 24 months of treatment with Anastrozole. This thesis focuses on evaluating the basic characteristics of the peripheral quantitative method: reproducibility; differences between loaded and non-loaded limbs; correlation with DXA measurements; effects of age, body mass index, and menopause; and differences in pQCT measurement parameters between healthy controls and breast cancer patients.

1.2.1 Thesis Objectives

The overall objective of this thesis is to contribute to the current literature concerning cortical and trabecular bone density and geometry at the non-dominant radius and tibia of postmenopausal women, with a specific focus on the effects of menopause and breast cancer therapy. This thesis is composed of five individual research projects, organized into complementary approaches, which present five distinct research outcomes. The first objective is to evaluate pQCT variables for the radius and tibia in pre- and postmenopausal women and to assess the *in vivo* reproducibility of density, content, and geometry measurements using the XCT-2000 pQCT scanner. The second objective is to evaluate the level of osteoporosis knowledge in a cohort of postmenopausal women, primarily women with a history of breast carcinoma. The third objective is to evaluate, via pQCT, the cross-sectional values for radial and tibial cortical and trabecular bone as well as calf muscle and fat cross-sectional area in healthy postmenopausal women and breast cancer patients prescribed Anastrozole. The fourth objective is to compare bone density and content measurements conducted via DXA with those by pQCT. The fifth objective is to assess the degree of change in cortical and trabecular bone measurement parameters in breast cancer patients after 12 and 24 months of treatment with Anastrozole.

1.2.2 Thesis Overview

The background and rational for investigating skeletal density and geometry via pQCT, with respect to the effects of menopause and breast cancer therapy on postmenopausal women are discussed in Chapter 2. Specifically, the purpose of the literature review is to contextualize the objectives of this thesis research by focusing on normal bone physiology and considering a range of factors that affect cortical and trabecular bone. Complete methodological descriptions of the materials, equipment and technology employed, bone parameters investigated, recruitment strategies, participant characteristics, and statistical analyses utilized, are provided in the material and methods chapter 3 of this thesis. Chapter 4 includes the results of the following studies: an examination of the *in vivo* reproducibility of pQCT-derived measurements, as well as factors affecting the reproducibility of these measurements; an assessment of the level of osteoporosis knowledge in postmenopausal women; the values of trabecular and cortical bone density and geometry at the radius and tibia in a cohort of healthy postmenopausal women and women with a history of breast cancer; correlation analysis of DXA measurements, serum vitamin, hormone, and mineral levels, and pQCT parameters in breast cancer subjects; and an examination of the rate of bone loss and its association with the initial volumetric bone mineral density as measured by pQCT in postmenopausal breast cancer patients after 12 and 24 months of treatment with Anastrozole. Furthermore, a review of the specific literature related to each study in question, the particular relevance of the findings for each experimental approach, and recommendations for future research are discussed in Chapters 5. A summation of the thesis is presented in Chapter 6.

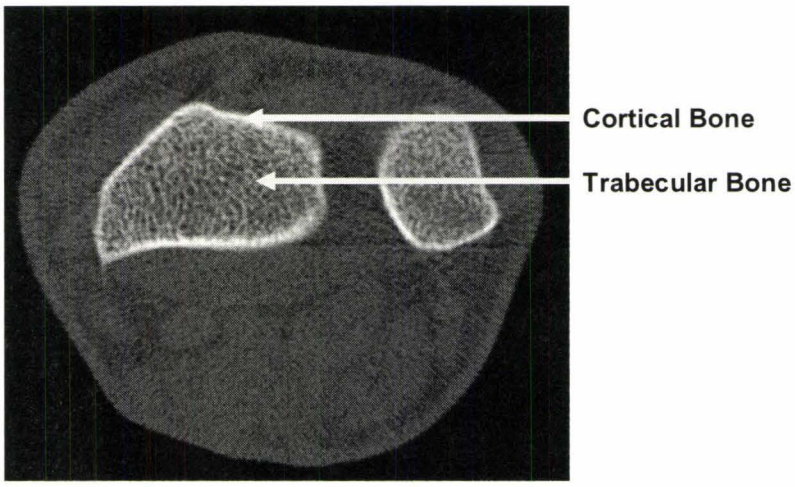
Chapter 2

LITERATURE REVIEW

2.1 Physiology of Bone

Bone tissue is physiologically optimized for mechanical support, stabilization of body shape, protection of various organs, locomotion, and participation in metabolism associated with mineral and hematopoietic homeostasis. The human skeleton is composed of two physically and biologically distinct structures: cortical and trabecular bone (Figure 1).

Figure 1: A pQCT Image of the Distal Radius Depicting the Cortical and Trabecular Bone Compartments



The external surface of the skeleton consists of cortical bone, also known as compact bone, which is a dense layer of calcified tissue. Towards the metaphysis and the epiphysis, the cortex becomes progressively thinner and the internal space becomes filled with thin calcified trabeculae. This is trabecular bone, also known as cancellous or spongy bone. Trabecular and cortical bone contain cells that are embedded in an organic and inorganic matrix. The organic matrix, or osteoid, is primarily comprised of collagen, which is responsible for the elasticity, flexibility and tensile strength of bone. The inorganic matrix of bone consists of

various calcium salts, mainly hydroxyapatite, $Ca_{10}(PO_4)_6OH_2$, which gives bone its rigidity, hardness, and strength in compression. These organic and inorganic components are arranged in a distinctive manner in the trabecular and cortical bone compartments, resulting in site-specific properties. Nevertheless, the cortical shell and the trabecular network act in unison *in vivo* in manners that are still largely not understood.

2.1.1 Cortical Bone

The basic unit of cortical bone is the osteon. Each osteon has a cylinder-shaped appearance and is built around an osteonic canal.(Raisz, 1999) This central canal contains one or two blood vessels. Rings of intercellular material, forming concentric lamellae, surround the osteonic canals. Embedded in this material are the individual osteocytes, which are located in small lacunae and are connected to other cells by radiating canaliculi. The canaliculi form intricate pathways throughout the bone, providing nourishment to osteocytes and removing waste products from these cells. Osteons are arranged so that the osteonic canals travel the length of the bone.(Raisz, 1999) Transverse Volkmann's canals connect the osteonic canals and provide communication between the bone surface and medullary cavity.(Raisz, 1999) Both the diameter and the thickness of the cortex have a dramatic impact upon the biomechanical integrity of the bone.(Oxlund *et al.*, 1993; Turner, 2002) Cortical bone is most abundant in the shafts of the long bones of the appendicular skeleton and is removed primarily by endosteal resorption and resorption within the haversian canals.

2.1.2 Trabecular Bone

Trabecular bone consists of trabeculae, which are an irregular array of bony plates that

resemble latticework. Trabecular bone is prominent near the ends of the long bones and in the vertebral bodies. Osteocytes are arranged within the trabeculae, and similar to cortical bone, they receive nutrients through small canalicular channels in the trabeculae, which extend from the bone cells to the surface of the trabeculae. Trabecular bone has an extensive surface area due to its large network of bony plates and spaces, and is metabolically more active than cortical bone for this same reason.(Dambacher *et al.,* 1998; Lau *et al.,* 1993; Riis *et al.,* 1996) In both males and females, trabecular compartments exhibit variability in density,(Ruegsegger *et al.,* 1991b) and can show marked changes during various skeletal disorders.(Boonen *et al.,* 1999; Gatti *et al.,* 1996; Ruegsegger *et al.,* 1984; Ruegsegger *et al.,* 1991b; Ruegsegger *et al.,* 1995)

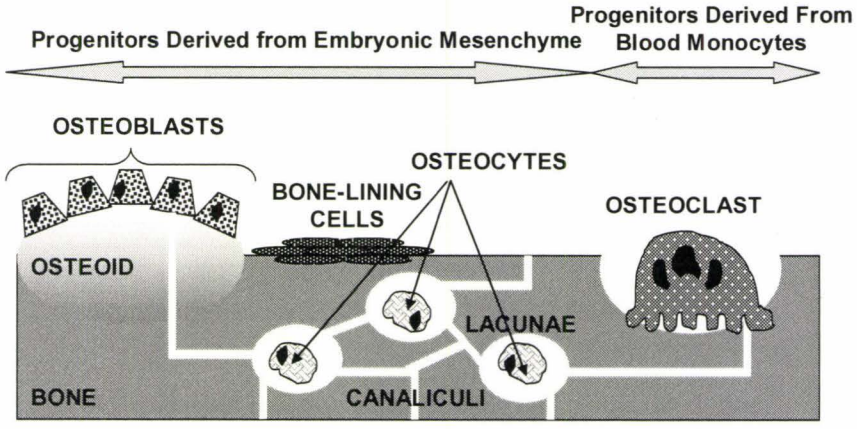
The orientation and connectivity of the collagen fibers in trabecular bone are arranged to accommodate the most constant loading stress exerted by the force of gravity.(Goulet *et al.,* 1994) Under normal loading circumstances, trabecular bone is strong and resilient when subjected to forces of compression at the ends of the bones. Trabecular failure, such as buckling and bending, occurs if there is a decline in the trabeculae perpendicular to the direction of the load. Strut strength is inversely related to the square of the unsupported length.(Bell *et al.,* 1967; Carter & Hayes, 1977) In terms of cancellous architecture, thick trabeculae are biomechanically less competent than an equivalent amount of bone arranged as more numerously connected thin trabeculae.(Kleerekoper *et al.,* 1985; Manolagas, 2000; Weinstein & Hutson, 1987) During the aging process, the loss of cancellous bone is not simply due to a generalized thinning of bone plates. Rather, there is a preferential loss of horizontal trabeculae,(Mosekilde, 1988; Thomsen *et al.,* 2002) caused by complete trabecular

perforation and fragmentation.(Kleerekoper *et al.,* 1985; Parfitt *et al.,* 1983) Vertebrae, which have a large proportion of trabecular bone, are most commonly the first sites to show bone loss. Fractures of the wrist and hip usually begin in the metaphyseal-epiphyseal regions of the bone, which depend heavily on the trabeculae to support loads.(Brown & Ferguson, 1978; McBroom *et al.,* 1985)

2.1.3 Bone Remodeling

Bone is constantly remodeled as a result of the coordinated actions of bone-forming osteoblasts and bone-resorbing osteoclasts (Figure 2).(Rodan, 1998) Their relative activities are under tight physiological control and are influenced by multiple endocrine and metabolic factors.

FIGURE 2: A GRAPHIC REPRESENTATION OF THE CELLULAR ORGANIZATION WITHIN THE BONE MATRIX



Osteoclasts are derived from blood monocytes. In trabecular bone, they are positioned in contact with a calcified bone surface and within shallow depressions called Howship's

lacunae, which are the result of their own resorptive activity.(Boskey, 1998; Roodman, 1996) In cortical bone they are located around the cylindrical-shaped osteonic canals. The most prominent features of the osteoclast are the distinctly large cell size, the multiple nuclei, and the deep folding of the plasma membrane in the area facing the bone matrix. The 'ruffled border' in the center is surrounded by a specialized ring of actin that delineates the osteoclast's area of attachment to the bone surface, thus sealing off the sub-osteoclastic compartment.(Tanaka *et al.,* 2005) The ability of the osteoclast to seal off this area of bone surface allows the formation of a microenvironment suitable for acidification and proteolytic digestion of the bone. The process of decalcification, and the breakdown of osteoid, results in the saucer-shaped Howship's lacunae on the bone surface. Bone resorption releases calcium from the bone and is an essential component of bone modeling and remodeling.

The osteoblast is the bone-lining cell primarily responsible for the production of the matrix constituents. Osteoblasts never function individually, but are always found in clusters of cuboidal-shaped cells (approximately 100-400 cells per bone-forming site) along the outer surface of bone.(Tanaka *et al.,* 2005) They are derived from local bone marrow stromal stem cells or connective tissue mesenchymal stem cells. Osteoblasts synthesize and secrete organic collagen-containing matrix, osteoid, on preexisting mineralized surfaces.(Tanaka *et al.,* 2005) Osteoid tissue is the layer of matrix formed by the osteoblast before it is calcified. Each osteoblast has a large nucleus, many mitochondria, and a well-developed Golgi apparatus.(Tanaka *et al.,* 2005) These organelles are associated with the secretory functions of the osteoblast. As bone formation slows, osteoblasts become incorporated into bone as osteocytes or remain on the surface as bone-lining cells. Osteoblastic activity occurs

continuously in all living bones; therefore, some new bone is constantly being formed.

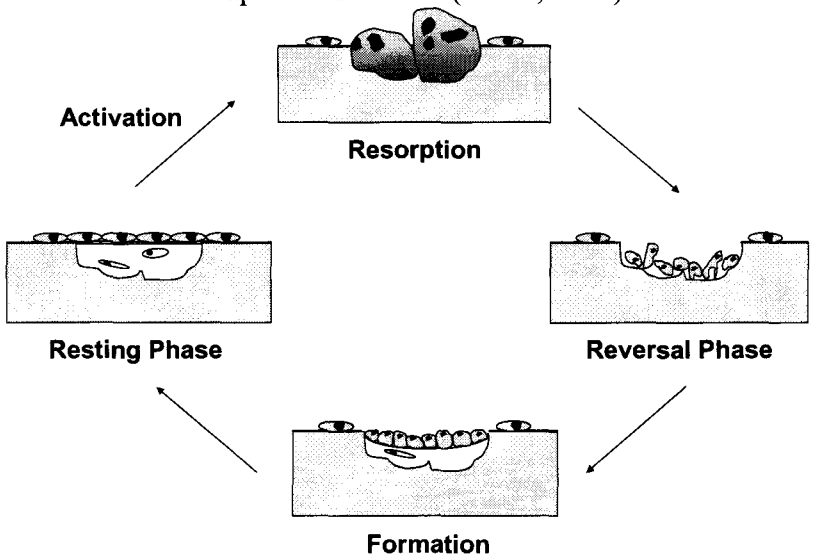
The osteocytes are cells that were originally osteoblasts which subsequently became embedded in the bone matrix that they produced and later became calcified. They are found within either the osteoid or the bony tissue in small osteocytic lacunae.(Franz-Odendaal *et al.,* 2006) Once osteocytes are entrapped, they lose their ability to secrete matrix. The osteocyte also develops numerous long fine canaliculi, rich in microfilaments, which form a network of communication with adjacent osteocytes and bone surface cells, such as osteoblasts, lining cells, and osteoclasts.(Franz-Odendaal *et al.,* 2006) These processes permeate the entire bone matrix and are organized during the formation of the matrix and before its calcification. The role of osteocytes is not clearly defined. These cells likely have a dual role within the bone; they act in a mechano-sensory manner by detecting and adapting to changes in the interstitial fluid flow produced by mechanical forces, and they communicate the need for focal repair of microdamage to lining osteoblasts on the bone surface in order to optimize bone mass and structure.(Qiu *et al.,* 2003)

When deprived of bone loading, osteocytes become hypoxic after twenty-four hours of disuse, indicating that mechanical loading may be essential to maintain nutrient supply and waste removal.(Dodd *et al.,* 1999) The fate of the osteocyte is to be phagocytized and digested together with the other components of bone during osteoclastic bone resorption, possibly as a result of osteocyte under-stimulation.(Burger & Klein-Nulend, 1999) Also derived from osteoblasts are the flat or elongated bone-lining cells which are found on the surface of the bone. Bone-lining cells are also known as resting surface cells and one

hypothesized function of these cells is the secretion of proteolytic enzymes.(Baron, 2003) By partial degradation of the mineralized surface, they prime the bone surface, enabling osteoclasts to begin bone removal or resorption.

Bone remodeling refers to the turnover of bone, the process whereby old bone is resorbed and replaced with new bone. This turnover is driven by both structural and metabolic needs and allows for bone development and growth, preventative maintenance of mechanical strength, and access to the skeletal stores of calcium and phosphorus for mineral homeostasis.(Tanaka *et al.,* 2005) In the normal adult skeleton, after the period of development and growth, bone formation occurs for the most part only where bone resorption has previously occurred. Therefore, the two processes are 'coupled' (Figure 3) and the site at which active bone remodeling takes place is known as a bone modeling unit (BMU).(Tanaka *et al.,* 2005)

FIGURE 3: THE BONE REMODELING PROCESS*
*Adapted from Baron.(Baron, 2003)



Remodeling also allows the microdamage of worn or injured bone to be replaced, further helping to maintain skeletal strength. Most bone turnover occurs at the bone surfaces, particularly at the endosteal surface where it interfaces with bone marrow. The duration of the complete remodeling cycle at each microscopic site requires approximately 6-9 months and the interval between successive remodeling events at the same location is approximately 2-5 years.(Parfitt *et al.,* 1996) The rate of turnover of the whole skeleton is about 10% per year. This is based on an average 4% turnover/year in cortical bone, which represents around 75% of the entire skeleton, and an average of 28% turnover/year in trabecular bone, which represents around 25% of the skeleton (0.75 x 4 = 3 and 0.25 x 28 = 7; 3 + 7 = 10).(Parfitt *et al.,* 1996)

The osteoblast-synthesized osteoid undergoes mineralization in two consecutive steps; a primary mineralization on the calcification front is followed by a slow process of secondary mineralization.(Meunier & Boivin, 1997) Primary mineralization generally begins 5-10 days after osteoid deposition and is typified by a rapid, linear rate of mineralization that proceeds until the remodeling cavity has been filled to 50-60% of the mineralization maximum. Following primary mineralization, the rate of mineralization slows and a phase of secondary mineralization begins; this progressively continues for a number of years.(Meunier & Boivin, 1997) Mineralization is rarely, if ever, complete and typically stabilizes around 90-95% of the maximum level.(Boivin & Meunier, 2002) Small losses in bone mass are observed over time in adults, which are thought to be the consequence of an imbalance between osteoclast and osteoblast activity, with the former removing more bone per BMU than is replaced by the latter. Besides an imbalance of resorption and formation within a BMU, further bone loss

may also occur as a result of an increase in the number of new BMUs. During times of high turnover, such as menopause, there is both an acceleration of active remodeling and a slightly greater discrepancy between bone resorption and formation, resulting in more significant bone loss.

2.2 Bone Density Measurement Techniques

Bone densitometry provides a quantitative assessment of the skeleton. Bone Mineral Density (BMD) is the most widely accepted surrogate index of bone mass and strength. Perhaps the most frequently assessed densitometry parameter at present is areal bone mineral density (aBMD), the mineral mass of bone per unit area of the two-dimensional projection image. This parameter can be measured by Dual Energy X-ray Absorptiometry (DXA) and is expressed as grams per square centimeter (g/cm^2). In contrast, quantitative computed tomography (QCT) measures volumetric bone mineral density (vBMD), expressed as grams per cubic centimeter (g/cm^3). These methods estimate bone mass on the basis of tissue absorption of photons derived from an x-ray source and are based on the principle that the attenuation of a beam of photons is related to tissue thickness and composition.

2.2.1 Dual Energy X-ray Absorptiometry (DXA)

DXA is currently considered the method of choice for the evaluation of bone mineral status of the skeleton in clinical practice and bone research. DXA utilizes two beams of different energies, thus allowing for corrections to be made regarding the attenuation of X-rays by soft tissues surrounding the skeletal site of interest.(Blake & Fogelman, 1998; Hagiwara *et al.,* 1994; Johnston *et al.,* 1991; Levis & Altman, 1998) The readings from absorptiometry are

calibrated for the amount of bone mineral content (BMC) measured over the area of bone scanned, thus DXA yields results based upon a weighted average of combined trabecular and cortical bone mass as aBMD of the skeletal site. DXA allows rapid measurement at both axial (vertebrae, femoral neck, total hip, and trochanter) and appendicular sites (distal radius, distal femur, proximal tibia, calcaneus, and phalanges).

The radiation dose with any single site DXA measurement is less than 10 microsievert (μSv) of ionizing radiation (Table 1).(Hagiwara *et al.,* 1994; Johnston *et al.,* 1991) The benefits of DXA include excellent precision, accuracy, short examination time, low radiation exposure, and the ability to predict fracture risk.

TABLE 1: STANDARD VALUES FOR EFFECTIVE RADIATION DOSE*
*Adapted from Schönau.(Schönau, 1998)

Type of Irradiation	Effective Dose (μSv)
DXA	<10
pQCT	<2
QCT	70-400
Natural background radiation per year	2400
Lateral X-ray film of the lumbar spine	700
Antero-posterior X-ray film of the lung	50
4 week's stay at an altitude of 2000 m	50
Return transatlantic flight	80

When referenced against *in vitro* phantoms, the accuracy and precision of DXA in measuring aBMD are useful, but the values are limited in the clinical setting for several reasons.(Ho *et al.,* 1990; Lilley *et al.,* 1991) Primarily, the 2-dimensional measurement obtained with DXA does not take into account the true bone size. This can lead to a diagnosis of osteoporosis in persons of petit stature, purely because their bones are smaller than that of the reference

population. Secondly, aortic calcification and artifacts such as metastases, compression fractures and osteophytes or bone pathologies such as scoliosis, kyphosis and vertebral collapse located at the image site can also heavily interfere with BMD measurement and can produce an erroneous interpretation of the results. Thirdly, BMD results from different instruments are not comparable or interchangeable because equipment calibration (*i.e.* adjustment for fat content, edge detection algorithms) depends on the specific device used. Fourth, the utility of the DXA-derived aBMD is restricted by the inherent planar nature of the measurement.(Genant *et al.,* 1996a) DXA is unable to discriminate between cortical and trabecular bone compartments, which are known to show different responses to aging, various diseases and treatments.(Boonen *et al.,* 1999; Gatti *et al.,* 1996; Rico *et al.,* 1994; Ruegsegger *et al.,* 1984; Ruegsegger *et al.,* 1991a; Ruegsegger *et al.,* 1991b; Ruegsegger *et al.,* 1995) For these reasons, a situation may occur where a redistribution of bone mineral takes place within an existing bone structure which may not be detected via DXA.(Cheng *et al.,* 1995) Furthermore, standard densitometric determinations of BMC and aBMD do not provide information concerning bone material quality or distribution. Hence, these measurements do not adequately assess bone mechanical competence in many instances. As a consequence of the above-noted limitations, some treatment effects on bone strength may remain concealed or be misinterpreted if studied with DXA alone.(Genant *et al.,* 1996a; Genant *et al.,* 1996b; Sievanen *et al.,* 1996)

2.2.2 Quantitative Computed Tomography (QCT)

QCT is a method of reconstructing a volumetric model of an object by producing a transverse cross-sectional image of the sample of interest via the use of multiple x-rays.(Morgan, 1983)

The object may be an entire body imaged transaxially by means of thoracic QCT, a limb imaged through peripheral quantitative computed tomography (pQCT), or a bone sample imaged via micro computed tomography (μCT). The sample is placed into a chamber in which an X-ray source rotates around its circumference. The rays are recognized by a ring of detectors located in the gantry. The attenuation of each x-ray beam represents the summed attenuations of each volume element, or voxel, through which the x-rays pass. The data is collected and processed by a computer, which contains an algorithm to reconstruct an image, represented by a matrix of CT numbers. The CT number is the linear attenuation coefficient expressed as an integer relative to the linear attenuation coefficient of water at the kilovoltage of the x-ray beam for each voxel of the image. Spatial resolution is defined by the number of voxels per unit volume, with greater resolution often limited *in vivo* due to increased levels of radiation exposure. The reconstructed image is then displayed, where each voxel is presented in a shade of gray relative to its CT number.(Morgan, 1983) A diagram of the appropriate positioning of a patient in a pQCT device, the location of the scan sites, and representative pQCT images of the radius and tibia are displayed in Appendices A through F.

QCT technology allows for the separate study of cortical and trabecular bone. pQCT was developed to provide simultaneous information on geometric properties and volumetric density, specifically for appendicular bone. Bone geometric properties include both the mass of the mineral and the cross-sectional area of bone, as well as the spatial arrangement of bone material at the skeletal site of interest. It has been suggested that trabecular bone density and bone quality parameters measured by pQCT may be a reasonable surrogate for measurements by histomorphometry,(Ito *et al.,* 1997) allowing for a non-invasive method of assessing bone

structure and geometry.(Augat *et al.,* 1998b; Louis *et al.,* 1995; Sievanen *et al.,* 1998) The XCT-series of pQCT bone scanners are fully automated systems for the determination of bone density at peripheral skeletal sites. These units work with a specially developed x-ray tube with a very small radiation output. The effective radiation dose with any single site measurement is less than 2 μSv of ionizing radiation. Table 2 depicts selected bone mass, density, and geometry parameters measured by pQCT.

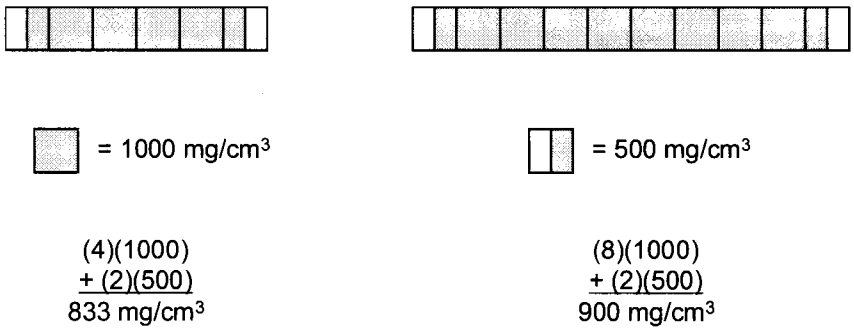
TABLE 2: SELECTED BONE QUALITY PARAMETERS MEASURED BY PQCT

Mass Indicators	▪ Volumetric Total BMC ▪ Volumetric Trabecular BMC ▪ Volumetric Cortical BMC
Density Indicators	▪ Volumetric Total BMD ▪ Volumetric Trabecular BMD ▪ Volumetric Cortical BMD
Geometry Parameters	▪ Total Area ▪ Cortical Area ▪ Polar Moment of Inertia ▪ Polar Moment of Resistance ▪ Polar Stress-Strain Index

In contrast to DXA, pQCT allows for direct density measurements of the metabolically active trabecular bone. This has spurred the use of pQCT in the research setting due to the fact that trabecular bone is a more sensitive indicator of slight treatment effects than cortical bone. Moreover, pQCT can give an actual description (*i.e.* shape and specific dimensions) of the cross-sectional geometry and bone composition (*i.e.* trabecular to cortical bone ratio) at a variety of skeletal sites in contrast to the planar and integral description of the same sites by DXA. pQCT can enhance our understanding of the relationships between bone density, bone composition, and bone strength with respect to demands on the loading environment and thus facilitate interpretation of the data obtained.(Beck, 1996; Genant *et al.,* 1994) In addition,

pQCT trabecular density measurements are largely independent of anthropometric measurements(Banks & Stevenson, 1986; Cann *et al.,* 1985; Compston *et al.,* 1988; Genant *et al.,* 1982; Karantanas *et al.,* 1991) whereas DXA is substantially affected by anthropometric parameters.(Carter *et al.,* 1992; Nielsen *et al.,* 1993) However, the strengths of pQCT as a powerful tool are countered by disadvantages: the equipment is expensive to obtain, rarely available for routine clinical use, and less precise than DXA, especially with measurements performed over time. Also, pQCT has not been shown to be more accurate than DXA in predicting fracture risk.(Bergot *et al.,* 2001) Fracture risk assessment based on t and z scores is currently most valid for DXA, whereas only limited data exist for quantitative CT. Furthermore, particularly in the case of thin cortices, the partial volume effect (Figure 4) can result in underestimated cortical bone measurement values.

FIGURE 4: ILLUSTRATION OF THE PARTIAL VOLUME EFFECT ON MEAN DENSITY VALUES*
*Adapted from Schönau.(Schönau, 1998)



Regarding the measurement of cortical thickness, if the cortical bone is thin, the partially filled-out voxels at the edges of the bone are responsible for the detection of falsely low densities. Overall, these disadvantages make pQCT, in practical terms, a less satisfactory

procedure than DXA. However, pQCT can greatly augment the information provided by DXA by presenting detailed, noninvasive assessment of the cross-sectional geometry and density of many peripheral skeletal sites. Therefore, pQCT has the potential to improve the diagnostic utility of densitometry in both the healthy and diseased skeleton.

2.2.3 Bone Biomechanics and Bone Quality

Although BMD remains the best available non-invasive assessment of fracture risk in routine clinical practice, many other skeletal characteristics also contribute to bone strength. 'Bone quality' is an umbrella term that encompasses several factors that influence bone strength and susceptibility to developing osteopenia or osteoporosis. Osteoporosis is usually multi-factorial in origin; in 2002, the National Institutes of Health modified the definition of osteoporosis to encompass:

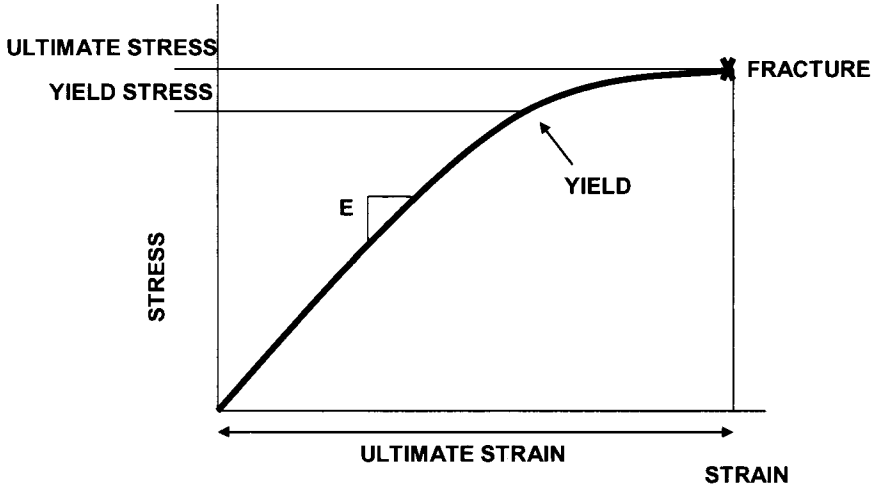
> "... a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength reflects the integration of two main features, bone density and bone quality."(Hellekson, 2002)

The recognition of bone quality and its incorporation into algorithms of fracture risk determination remains the subject of continuing translational research. The strength of bones results from a combination of two complimentary properties; these include bone material and geometry.(Martin, 1991) Bone material properties concern the intrinsic stiffness and strength of the solid bone substance, regardless of mass, size, shape and macrostructure. Bone geometric properties concern the whole bone macro-architecture. In cancellous bone, bone geometry accounts for the spatial distribution, thickness, connectivity and microfractures of the trabecular network. In cortical bone it accounts for the cortical cross-sectional thickness, perimeters, area, and moments of inertia concerning bending and torsion. BMC, which is the

amount of mineralized bone tissue present, is the manifestation of these properties. Mass does not influence bone strength directly; rather, changing the distribution of BMC can change the ability of bone to resist bending and torsion and thus determine bone strength.(Marshall *et al.,* 1996; Ott, 1993; Wilkin, 1999)

Bone tissue receives and responds to mechanical signals by local adaptation to the functional loading environment. When stress is applied to any material, including bone, it exhibits strain. The force per unit area is stress (σ = Force/Area) and is reported in pascals (Nm^{-2}). Strain is a dimensionless unit formally defined as the amount of deformation in the material relative to its original length ($\epsilon = \Delta L/L$).(Burr & Turner, 2003) The stress strain curve (Figure 5) is obtained by increasing force to a bone specimen until it breaks.

FIGURE 5: THE STRESS-STRAIN CURVE FOR BONE TISSUE.

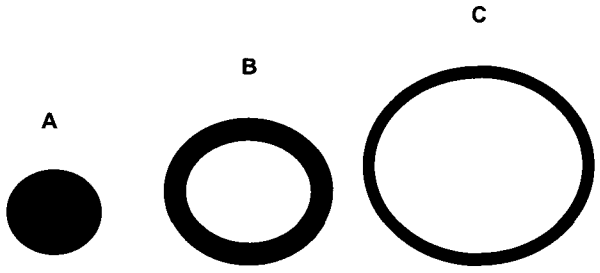


When a material is pulled, it gets longer (tensile strain), and when pushed together, the material shortens (compressive strain). Shear strain, the angle measured in radians, arises when layers of a material slide against another, as might occur with torsion or bending. The slope of the linear region of the stress-strain curve defines Young's modulus (E) of the material or stress/strain.(Burr & Turner, 2003) This represents material stiffness; the greater the slope, the more stiff the material. A gentle slope indicates a more compliant material. The stress at the peak of the curve is the ultimate stress, which is one measure of strength.(Burr & Turner, 2003) Another measure of strength is the stress at the yield point. The strain from the yield point to failure (post-yield strain) is a measure of brittleness.

An estimation of bone strength requires knowledge of the material and architectural properties of bone as well as its loading conditions.(Chevalier *et al.,* 1992; Dalstra *et al.,* 1993; Vesterby *et al.,* 1991; Wallach *et al.,* 1992) Low BMD at the hip, spine and wrist are significantly associated with the risk of osteoporotic fractures.(Cummings *et al.,* 1990; Hui *et al.,* 1990; Melton *et al.,* 1993; Seeley *et al.,* 1991) However, considerable overlap exists in BMD values between fracture and non-fracture subjects.(Cann *et al.,* 1985; Leslie *et al.,* 2003; Miller *et al.,* 2002; Schuit *et al.,* 2004; Siris *et al.,* 2001) In particular, micro-structural alterations correlate with, but appear to outstrip, changes in bone quantity.(Goldstein *et al.,* 1993; Martin, 1993; Mosekilde, 1989; Mosekilde & Mosekilde, 1990; Snyder *et al.,* 1993) Much of the information regarding bone architecture has been obtained from direct measurement of cadaveric specimens.(Martin & Atkinson, 1977; Meema, 1963; Ruff & Hayes, 1982; Ruff & Hayes, 1988) For example, Myers *et al.* found that the *in vitro* failure force of the distal radius correlated with the distal radius width, cross-sectional area and

principle area moments of inertia, but not with BMC or BMD.(Myers *et al.,* 1993) Evidence from rat femora also showed that the strength and stiffness of the integrated diaphyses depends on both body weight and the cross-sectional moment of inertia, but not on BMD.(Ferretti *et al.,* 1993) Moreover, mechanical failure of the proximal femur and distal radius has been shown to depend not only on the total bone mass but also more specifically on the thickness and geometric properties of the cortical shell.(Augat *et al.,* 1996; Laval-Jeantet *et al.,* 1983; Louis *et al.,* 1995) The maximal compressive strength of the radius was found to yield a significantly positive correlation with cortical thickness and cortical BMD.(Louis *et al.,* 1995) Interestingly, decreased BMD in some cases can be associated with maintained or increased strength, through compensatory structural and geometrical adaptations such as the addition of a small amount of bone to the periosteal surface. Bone added to the periosteal surface can increase bone strength, even though the absolute bone volume and BMD are unchanged. The diagram in Figure 6 illustrates this concept.

FIGURE 6: SCHEMATIC DIAGRAM OF THE EFFICIENT DISTRIBUTION OF BONE MATERIAL DURING PERIOSTEAL APPOSITION*
*Adapted from Burr and Turner.(Burr & Turner, 2003)



1.77	1.77	1.77	AREA (CM^2)
0.25	0.64	1.13	SECOND MOMENT OF AREA (CM^4)

Bone area reflects BMD, and the second moment of the area is an indication of resistance to bending.(Burr & Turner, 2003) The bone structure in B has a 2.5-fold greater second moment of area than a solid structure A, although both have the same bone area.(Burr & Turner, 2003) The increased diameter and decreased cortical thickness in C, causes the second moment of area to increase further to 4.5-fold greater than A. Therefore, bone modeling by periosteal apposition reduces compressive stress by distributing loads on a larger area and partly maintains bending strength.(Duan *et al.,* 1999; Duan *et al.,* 2001) As endosteal bone loss proceeds, concurrent periosteal apposition increases cross-sectional bone area, thus reducing the stress on the bone and partially offsetting bone loss so net bone loss is reduced. Periosteal apposition of bone with age serves a biomechanically important function by compensating for reduced tissue properties or reduced bone volume in men, but this compensation has not been shown to offset the larger loss of bone that occurs in modern populations of women.(Duan *et al.,* 2001; Manolagas, 2000; Martin & Atkinson, 1977)

Architectural parameters have been devised that allow for the calculation of the strength of a structure from the amount and distribution of the raw material. Examples include the polar moment of inertia (PMI) and the section modulus.(Turner & Burr, 1993) The PMI reflects the flexural and torsional strength of diaphyseal bone and is a measure of the distribution of material around the central axis of a specimen.(Turner & Burr, 1993) Maximum and minimum moments of inertia represent the distribution of material around the axes of the maximum and minimum bending strengths, respectively. The section modulus is a closely related parameter; a higher section modulus means that mechanical failure occurs at higher loads.(Martin, 1991) The elastic modulus is a measure of the intrinsic stiffness of the

material during bending. The shear stress created in a bone by torque is inversely related to the PMI.(Turner & Burr, 1993) Thus, in a bone with a higher PMI, the same torque will result in smaller shear stress than in a bone with a lower PMI. Arguably, the most important parameter describing the material quality of bone is the elastic modulus (Young's modulus; *E)*, which represents the stiffness of a material. It is known that bone strength is determined by both the material properties or stiffness, described by *E*, and the spatial distribution of that material, the geometry.(Currey, 1988; Ferretti *et al.,* 1993; Turner & Burr, 1993) It has been shown experimentally that the vBMD of cortical bone in the narrow physiological range has an approximate linear relationship with *E* (Burstein *et al.,* 1975; Currey, 1969; Currey, 1988; Ferretti, 1995; Ferretti *et al.,* 1996; Martin, 1991; Rho *et al.,* 1993) and vBMD can be measured by pQCT with high precision and accuracy.(Ferretti *et al.,* 1995; Ferretti, 1995) Moreover in an attempt to predict bone strength non-invasively, a Bone Strength Index (BSI) and a more elaborate Stress Strain Index (SSI) have been proposed, which take into account both density and geometry data derived from pQCT.(Ferretti, 1995) The BSI has been found to be a strong predictor of the torsion and bending strength of bones in mechanical testing of human cadaver radii and small animal bones.(Augat *et al.,* 1996; Ferretti *et al.,* 1993) The SSI, calculated as the product of section modulus and cortical vBMD normalized to the maximal physiological cortical vBMD of human bones, has been shown to provide a good estimate of the mechanical strength of human radii.(Augat *et al.,* 1998d) With current pQCT technology it is possible to calculate the moments of inertia and resistance, as well as SSI values directly from cross-sectional pQCT images.

2.2.4 The Functional Muscle-Bone Unit

In addition to bone strength and its biological components, pQCT can also measure the cross-sectional area of regional muscles.(Rittweger *et al.,* 2000) This provides valuable information concerning muscle-bone interactions that are essential for determining the bone mechanostat condition. The mechanostat theory proposes that bones adapt their strength to the high dynamic load of muscle force and thus there is a close relationship between bone strength and muscle force or size.(Frost, 1987; Rauch & Schoenau, 2001) According to the mechanostat theory, bone modeling and remodeling alters bone mass and architecture in order to keep the level of strain on bone within an operational range. The phrase 'Bone Muscle Strength Index' (BMSI) has recently been coined to describe this quantitative relationship.(Rittweger *et al.,* 2000)

Regional muscle strength is important for determining the strength of the whole bone regardless of sex, age, or body habitus. Muscular contraction routinely puts large loads on the skeleton, therefore, bone stability has to be adapted to muscle force. Muscle attachments are generally close to the joint so they must generate large forces on bone for the bone to function as a lever. When bouts of resistance exercise are repeated, increases in muscle protein synthesis in the exercised muscles result in an increase in myofibrillar cross-sectional area.(Chesley *et al.,* 1992) Long bones tend to change their geometry rather than BMC to meet increased load demands; thus, in a similar manner, muscles respond to overload by increasing the formation of muscle protein. Since muscle force scales with muscle size,(Ruff, 2003) muscle cross-sectional area can be used as an index of habitual local skeletal load. Muscle strength and lean mass have been reported to be positively associated with bone mass

of adjacent skeletal segments(Aloia *et al.,* 1991) with 10-20% of the variance in BMD attributed to physical activity.(Vico *et al.,* 1995) Interestingly, bicep strength best predicts hip BMD whereas grip strength independently predicts BMD of the lumbar spine and radius.(Snow, 1996) It is intriguing that Marquis *et al.* found muscle mass to be a better predictor of mortality than body weight in stable COPD patients.(Marquis *et al.,* 2002) In the study, a mid-thigh cross-sectional muscle area of ≤ 70 cm^2 was seen to increase the risk of death four-fold, independently of the influence of other prognostic variables. (Marquis *et al.,* 2002) Given the strong interrelationship between muscle force and bone strength, diagnostic indices that quantify these relations may turn out to be valuable tools for evaluating bone quality.

2.3 Pathophysiology of Postmenopausal Bone Loss

Approximately two-thirds of postmenopausal bone loss can be ascribed to the cessation of ovarian function during menopause, and the remaining one-third can be attributed to estrogen-independent, multifactorial, involutional mechanisms.(Riggs *et al.,* 1981) Senescence contributes to this slow phase of bone loss through: (*i*) secondary hyperparathyroidism caused by age-related decrease in the ability to adapt to a lower calcium intake by increasing intestinal calcium absorption; (*ii*) increased renal calcium excretion; (*iii*) impaired vitamin D metabolism; (*iv*) impaired osteoblast recruitment and function.(Dick *et al.,* 1996; Eastell *et al.,* 1988; Garnero *et al.,* 1996a; Garnero *et al.,* 1996b; Gennari *et al.,* 2002; Melton *et al.,* 1997) During aging, ever-diminishing and architecturally disrupted bone due to estrogen deficiency predisposes elderly women to fractures brought on by minimal trauma, at sites rich in trabecular bone such as the vertebra and wrist.(Courtney *et al.,* 1996)

In addition, after menopause, bone tissue becomes less able to sustain further strain once it has been damaged.(McCalden *et al.,* 1993) This is referred to as Type I postmenopausal osteoporosis.(Riggs *et al.,* 1998; Riggs & Melton, 1986) Involutional osteoporosis also contributes to a decline in bone density and bone quality, and confers increased skeletal fragility and propensity to fracture at sites containing both cortical and trabecular bone. This is referred to as Type II senile osteoporosis.(Riggs & Melton, 1986)

Peak bone mass is the maximum mass of bone achieved by an individual at skeletal maturity, typically between the ages of 25 and 35. Several factors determine an individual's peak bone mass, a few examples are listed in Table 3.

TABLE 3: FACTORS THAT DETERMINE PEAK BONE MASS

Factors	Description
Genetics	Demonstrated in studies of twins and studies establishing a strong relationship between the bone mass of mothers and daughters
Gender	Women accumulate less skeletal mass than men during growth, particularly during puberty, resulting in smaller bones with thinner cortices and smaller diameter
Ethnic Origin	People of African origin generally have higher bone mass than those of northern European origin or Asian origin
Multiple Genes	For example, the vitamin D receptor allele, estrogen receptor genes, and collagen receptor genes may be associated with the development of peak bone mass
Nutritional Factors	Calcium, vitamin D, and protein intake all affect bone mass
Hormonal Factors	The timing of puberty and menopause are important; estrogens play a key role in bone mass acquisition during puberty and in bone mass maintenance throughout the reproductive years
Weight-Bearing Exercise	Muscle pulling on bone builds denser, stronger bones
Environmental Factors	For example, tobacco and alcohol consumption, prolonged immobilization, and glucocorticoid use exert negative impacts on bone

Cross-sectional studies suggest that peak bone mass may be achieved as late as the third and possibly the fourth decade at some skeletal sites.(Halioua & Anderson, 1990; Tylavsky *et al.,* 1989) Bonjour *et al.* have proposed that predominantly trabecular sites such as the spine achieve peak bone mass during the adolescent years, prior to cortical sites such as the shaft of long bones.(Bonjour *et al.,* 1993) It is peak bone mass that strongly predicts fracture risk in later life.(Goldstein *et al.,* 1993; Kelly *et al.,* 1993; Krall & Dawson-Hughes, 1993; Luckey *et al.,* 1989)

After peak bone mass is attained, bone mass remains relatively stable in women until menopause (around the age of 45-55). This is followed by an accelerated bone loss of 25-30% of the skeletal mass over a period of 5-10 years, and subsequently a slower phase(Stepán *et al.,* 1987) with stable bone loss of 0.5-1% occurring per year.(Nguyen *et al.,* 1995) The rate of loss is highest in the first 5 years after menopause(Ahlborg *et al.,* 2001) and is associated with a rapid deterioration of bone mass by as much as 3% per year.(Riggs *et al.,* 1998) In the years after menopause the annual decrease in the trabecular BMD in women was found to be 3.1% compared with 1.4% for the total BMD,(Muller *et al.,* 1989) whereas cortical BMD appeared to be relatively stable throughout various age groups.(Gatti *et al.,* 1996; Hangartner & Gilsanz, 1996; Ruegsegger *et al.,* 1991a; Ruegsegger *et al.,* 1991b) Overall, a woman can expect to lose about 35% of her cortical bone and 50% of her trabecular bone as she ages.(Mazess, 1982; Riggs *et al.,* 1981; Smith *et al.,* 1975) The risk of fragility fracture in postmenopausal women is determined mainly by a low BMD.(Cummings *et al.,* 1993; Marshall *et al.,* 1996; Stone *et al.,* 2003) There is, however, quite a substantial overlap of BMD values between fracture cases and controls because of the multiple

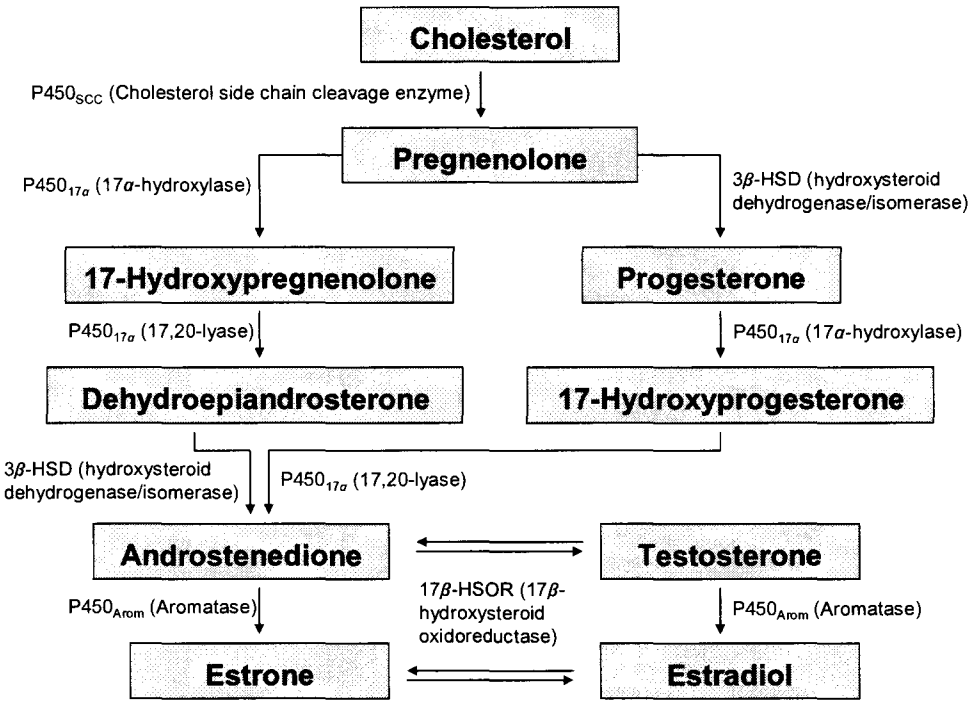
determinants of skeletal fragility.(Christiansen *et al.,* 1990; Melton *et al.,* 1993; Riis *et al.,* 1996) Furthermore, the normal ranges for BMD vary for different skeletal sites as well as with the age, gender, and race of the patient.

2.3.1 Estrogen and Bone

In the late 1940s, the importance of menopause with cessation of ovarian function and estrogen deprivation in the evolution of bone fragility was first recognized. A great deal of evidence has since documented the important role of estrogen in preserving skeletal integrity in women.(Christiansen *et al.,* 1980; Ettinger *et al.,* 1985; Genant *et al.,* 1982; Lindsay *et al.,* 1976; Riggs *et al.,* 1981) Estrogens are sex steroids that affect not only the reproductive organs, but also many other tissues including the brain, cardiovascular system and bone. The two main estrogens are estrogen (E1) and estradiol (E2); the third form, estriol (E3), is produced during pregnancy. All estrogens share an 18-carbon skeleton derived from cholesterol and the immediate precursors are C19 androgens.

The enzyme, $P450_{AROM}$ complex (aromatase), converts androgens to estrone and estradiol *in situ*. Intracrine estrogen production, via aromatase, becomes the only source of estrogens after menopause and therefore governs the degree of residual estrogen available. In postmenopausal women, the majority of the residual levels of circulating estrogen are derived from androgens which are secreted by the ovaries and the adrenal cortex and converted to estrogens in peripheral tissue, such as fat and muscle. Figure 7 illustrates the pathway of estrogen biosynthesis in the ovary and peripheral tissues.

FIGURE 7: THE PRINCIPLE PATHWAY OF STEROID HORMONE BIOSYNTHESIS IN THE OVARY AND THE CONVERSION OF ANDROGENS IN THE PERIPHERAL TISSUES



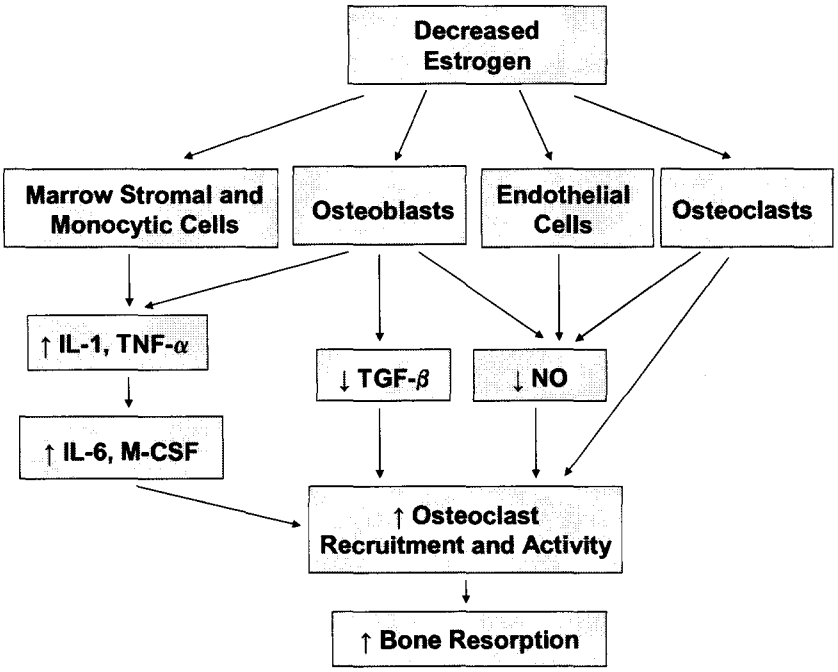
The precise mechanisms through which estrogen deficiency causes an increase in bone loss are not fully understood. Estrogens act on bone tissue both directly and indirectly. Indirectly, estrogen modulates the production and effect of calciotropic hormones (parathyroid hormone and vitamin D), promoting calcium retention and maintaining optimal calcium concentrations at mineralization sites. Directly, estrogen acts through 2 receptors: estrogen receptor alpha and beta (ERα and ERβ). Some studies suggest that the effects of estrogen signaling through ERα and ERβ are in opposition, while other studies propose that activation of these 2 receptors have similar effects on bone.(Sims *et al.,* 2002; Windahl *et al.,* 2001) However, ERα appears to be the primary mediator of estrogen's actions on the skeleton.(Lee *et al.,*

2003) with ERs present in both osteoblasts(Bord *et al.,* 2001; Eriksen *et al.,* 1988; Komm *et al.,* 1988) and osteoclasts.(Oursler *et al.,* 1991b)

In aging adults, the number of osteoblasts recruited to erosion surfaces is decreased as is their functional capacity, resulting in a decreased rate of bone formation.(D'Ippolito *et al.,* 1999) Estrogen promotes the differentiation of multipotent mesenchymal stem cells toward the development of osteoblasts in preference to adipocytes(Jilka *et al.,* 1996; Kajkenova *et al.,* 1997; Okazaki *et al.,* 2002), increases osteoblast proliferation,(Fujita *et al.,* 2002) and increases the production of a number of osteoblast proteins (*i.e.* insulin like growth factor-1 [IGF-1], and type 1 procollagen,(Ernst *et al.,* 1989) transforming growth factor beta [TGF-β],(Oursler *et al.,* 1991a; Yang *et al.,* 1996) and bone morphogenic protein-6 [BMP-6]).(Rickard *et al.,* 1998) Furthermore, estrogen exerts antiapoptotic effects on osteoblasts and osteocytes.(Gohel *et al.,* 1999; Weinstein & Manolagas, 2000) Specifically, estrogen deficiency promotes osteoblast apoptosis *in vitro* and *in vivo* by 2- to 3- fold, an effect seemingly mediated by TGF-β.(Hughes *et al.,* 1996) Thus, estrogen tends to have an anabolic effect on osteoblast cells, and may also be important in maintaining osteocyte function.(Tomkinson *et al.,* 1997) The increase in osteocyte apoptosis following estrogen deficiency could further weaken the skeleton through impairment of the osteocyte-canalicular mechano-sensory network.(Weinstein & Manolagas, 2000) Interestingly, recent *in vitro* studies with human osteoblastic cells indicate that OPG production is stimulated by estrogen, suggesting that this cytokine may also play an important role in the antiresorptive action of estrogen on bone.(Hofbauer *et al.,* 1999) In light of the fact that mesenchymal cell differentiation and osteoclastogenesis are tightly linked, stimulation of mesenchymal cell

differentiation toward the adipocyte lineage after sex steroid loss may be the first event that ensues after hormonal change, and increased osteoclastogenesis and bone loss might be downstream consequences of this change.(Weinstein *et al.,* 1997) Figure 8 depicts the effect of a decreased estrogen level on various cells in the bone microenvironment.

FIGURE 8: PATHWAYS BY WHICH ESTROGEN LOSS LEADS TO BONE RESORPTION*
*Adapted from Reid.(Reid, 2003)



In addition to increased bone remodeling, loss of systemic sex hormones leads to an increased number of resorption cavities and the increased depth of resorption lacunae.(Eriksen *et al.,* 1999; Mosekilde, 1990; Parfitt *et al.,* 1996) This deeper erosion may cause separation and disconnection of trabeculae and the removal of some cancellous elements entirely, leaving no template for new bone formation. Concurrent loss of cortical bone occurs due to the enlargement and coalescence of the subendocortical spaces, causing cortical thinning. The

net loss of trabecular connectivity translates into deterioration in trabecular architecture and decreased overall bone strength. This deeper erosion can now be explained by evidence that estrogen acts on mature osteoclasts to decrease their activity and promote their apoptosis.(Hughes *et al.,* 1996; Kameda *et al.,* 1997; Parfitt *et al.,* 1996) Consequently, loss of estrogen prolongs the lifespan of osteoclasts.(Fyhrie & Schaffler, 1994; Parfitt *et al.,* 1996)

While estrogen acts on cells of the osteoblastic lineage, its effects on bone are also dependent on cells of the hematopoietic lineage, including osteoclast precursors, mature osteoclasts, and lymphocytes. Data derived primarily from studies in mice indicate that loss of estrogen up-regulates the production and activation of several cytokines, such as IL-1 and IL-6,(Lin *et al.,* 1997; Manolagas, 1998; Manolagas & Jilka, 1995) Macrophage Colony Stimulating Factor [M-CSF],(Srivastava *et al.,* 1998; Srivastava *et al.,* 1999) and Tumor Necrosis Factor-α [TNF-α],(Pfeilschifter *et al.,* 2002; Srivastava *et al.,* 1998) all of which are responsible for osteoclastogenesis and osteoblastogenesis.(Jilka, 1998; Manolagas & Jilka, 1995; Pacifici, 1996; Pacifici, 1998) There is also an increase in the sensitivity of osteoclasts to these cytokines.(Sunyer *et al.,* 1999) The cytokines can have multiple effects on the osteoclast lineage, including the induction of osteoclast differentiation, maturation, stimulation of inactive osteoclasts to resorb bone, and inhibition of osteoclast apoptosis.(Pacifici, 1996) Estrogen decreases production of each of these cytokines *in vitro*(Girasole *et al.,* 1992; Jilka *et al.,* 1992; Pacifici *et al.,* 1989) and *in vivo*,(Rogers & Eastell, 1998) and may also modulate levels of receptors for IL-1.(Sunyer *et al.,* 1999) Several studies have shown that the level of expression of IL-6 receptors (IL-6Rα and gp130) are elevated in estrogen-deficient mice and rats as well as in humans, in the bone marrow and in the peripheral blood.(Bismar *et al.,*

1995; Cheleuitte *et al.,* 1998; Kassem *et al.,* 1996; Miyaura *et al.,* 1995; Spelsberg *et al.,* 1999)

Moreover, animals that lack the ability to synthesize or respond to IL-1, IL-6, or TNF-α, do not incur bone loss following an ovariectomy.(Jilka, 1998; Kimble *et al.,* 1995; Lorenzo *et al.,* 1998) An increase in osteoclast numbers and the resultant bone loss after ovariectomy is counteracted by blockers of IL-6(Jilka *et al.,* 1992) and IL-1 or TNF-α.(Kimble *et al.,* 1995) The reduction in bone resorption by estrogen may be further diminished by an increase in levels of nitric oxide (NO), which is a potent inhibitor of osteoclast differentiation and bone resorption.(Yang *et al.,* 1996) Estrogen may also help to regulate the release of systemic factors, including growth hormone.(Friend *et al.,* 1996) Another important characteristic of the cytokines that regulate osteoclastogenesis is their ability to amplify one another's synthesis in a cascade-like fashion. IL-1, IL-6 and TNF-α not only induce their own synthesis but TNF-α strongly synergizes with IL-1 to stimulate both TNF-α and IL-6.(Jilka, 1998) Because of this interdependent production of IL-1, IL-6 and TNF-α, a small change in one cytokine following estrogen loss leads to a dramatic increase in the level of all three cytokines.(Jilka, 1998)

Thus, the effects of estrogen on the production of cytokines and growth factors within the bone marrow micro-environment may act together with its direct effects on bone cells to modulate both bone resorption and bone formation. The imbalance between bone resorption and formation that ensues after loss of sex steroids can be explained through the dual augmentation of the survival of the bone-resorbing cells and simultaneous shortening of the

life span of the bone-forming cells. Additionally, the accumulation of apoptotic osteocytes, caused by the loss of estrogen, could increase bone fragility even before significant loss of bone mass, because of the impaired detection of microdamage and inefficient repair of substandard bone.

2.3.2 Postmenopausal Trabecular and Cortical Bone

All established techniques of bone mineral densitometry have detected accelerated bone loss in postmenopausal women, albeit at different rates according to the skeletal site measured (*i.e.* appendicular versus axial) and skeletal envelope (*i.e.* cortical versus trabecular). Bone loss associated with advanced age and estrogen deficiency in women is accompanied by a disturbance of bone micro-architecture.(Parfitt *et al.,* 1983; Parfitt, 1984; Parfitt, 1987) During aging, bone resorption on the endocortical, intracortical and trabecular surfaces reduces the amount of bone as trabeculae thin and disappear, and as cortices thin and become porous.(Seeman, 2002) Late in life, the total surface available for bone remodeling moves from the trabecular to the cortical compartment. Endocortical and intracortical remodeling increase and bone loss comes primarily from cortical bone since the surfaces within cortical bone increase due to increased intracortical porosity.(Gowen *et al.,* 1999) Thus, cortical bone becomes 'trabecularized'.

Women and men lose similar amounts of trabecular bone during aging.(Seeman, 2001) However, trabecular thinning predominates in men, while loss of connectivity dominates in women.(Aaron *et al.,* 1987) In the first years following the menopause, annual losses measured in spinal trabecular bone with quantitative CT are approximately 4-5%(Banks &

Stevenson, 1986; Compston *et al.,* 1988; Genant *et al.,* 1982; Karantanas *et al.,* 1991) and in spinal integral bone with DXA are approximately 2-3%.(Luisetto *et al.,* 1993; Ribot *et al.,* 1988) Over the entire lifetime from young adult to elderly, the average annual rates of spinal trabecular bone loss documented with quantitative CT are 1.2-1.8% in women and 0.8-1.4% in men with a net result of elderly women having 10-30% lower trabecular BMD than elderly men and 40-50% lower trabecular BMD than young women.(Banks & Stevenson, 1986; Compston *et al.,* 1988; Genant *et al.,* 1982; Karantanas *et al.,* 1991)

2.4 Breast Carcinoma and Bone Health

Breast carcinoma (BC) is the most common cancer in women accounting for one-third of all new cancer cases.(Jemal *et al.,* 2005) Fortunately, progress in BC detection and therapy has resulted in an increasing number of women who are long-term survivors of the disease. Patients with solid tumors, as with BC, are at risk of developing skeletal metastases and may experience accelerated bone loss as a result of their underlying malignancy, antineoplastic treatments, and associated co-morbid conditions (including malnutrition, loss of muscle mass and immobilization). Treatment with chemotherapeutic agents such as doxorubicin and methotrexate, leads to decreased trabecular bone volume and decreased bone formation in rats.(Delmas & Fontana, 1998) A recent study in dogs showed that doxorubicin, cisplatin, and ifosamide slowed the mineral apposition rate in cancellous bone.(Virolainen *et al.,* 2002) Chemotherapy may, therefore, exert direct deleterious effects on bone turnover, hindering bone formation. Furthermore, infiltration by malignant cells themselves may increase osteoclastic activity by promoting the release of TGF-α or -β.(Lamont & Lauderdale, 2003) Patients with cancer but without bone metastases have been shown to have increased bone

resorption as indicated by biochemical markers of bone turnover.(Delmas & Fontana, 1998) Overall, more research is required to investigate the actual effects and clinical significance of adjunctive chemotherapy and supportive medications on the bones of BC patients.

Approximately 70% of breast tumors express the ER(Harvey *et al.,* 1999) and both endogenous and exogenous estrogen have been implicated in the pathogenesis of BC.(Clemons & Goss, 2001) Relationships between body mass index (BMI), endogenous estrogen levels and the risk of BC have been reported.(Cauley *et al.,* 1989; Ganry *et al.,* 2004; Hall *et al.,* 1999; La Vecchia *et al.,* 1997; Vatten & Kvinnsland, 1990) Adult bone density and BC risk both reflect lifetime estrogen exposure. Moreover, BMD may be considered a surrogate marker for long term exposure to endogenous estrogen.(Cauley *et al.,* 1986) This raises the possibility that BMD measurements, in combination with other risk factors, could be predictive of women at high risk for BC who might benefit from preventive actions like BC screening. However, the inverse correlation between the risk of osteoporosis and BC continues to be investigated. Some epidemiological studies have found that women with osteoporotic fractures were less likely than women without fractures to develop subsequent breast cancer(Ganry *et al.,* 1999; Newcomb *et al.,* 2001; Olsson & Hägglund, 1992; Persson *et al.,* 1994) and a higher BMD was associated with greater risk of BC in the Study of Osteoporotic Fractures(Cauley *et al.,* 1996; Zmuda *et al.,* 2001) and the Dubbo Osteoporosis Epidemiology,(Nguyen *et al.* 2000) Epidemiologie do I'Osteoporose (EPIDOS)(Ganry *et al.,* 2004) and Framingham(Zhang *et al.,* 1997) studies. Findings from the Study of Osteoporotic Fractures suggest that women with the highest BMD had a 2.0-2.5 fold increase in the risk of BC compared with women with the lowest BMD.(Cauley *et al.,* 1996; Zhang *et al.,* 1997;

Zmuda *et al.,* 2001) Zmuda *et al.* showed that the magnitude of the association between BMD measurements taken at multiple skeletal sites and BC risk was greater for women diagnosed with advanced-stage disease than for women diagnosed with early-stage disease(Zmuda *et al.,* 2001). In the NHANES follow up,(Nelson *et al.,* 2002) the Fracture Intervention Trial (FIT)(Buist *et al.,* 2001) and the Rotterdam study,(van der Klift *et al.,* 2003) this association was determined to be weak or inconclusive. Most recently a large (n=15,254) case-control study of women in the San Francisco mammography registry, concluded that BMD was not a strong risk factor for the development of BC for that study population.(Kerlikowske *et al.,* 2005)

Research into the risk of hip fracture in breast cancer survivors is also inconsistent, suggesting either an increased(Adami *et al.,* 1990) or reduced risk.(Lamont & Lauderdale, 2003) Several small studies have found low bone density among postmenopausal breast cancer survivors(Lindsey *et al.,* 2002; Twiss *et al.,* 2001) and accelerated bone loss after chemotherapy for BC,(Bruning *et al.,* 1990; Greep *et al.,* 2003; Headley *et al.,* 1998) suggesting an increased risk for fractures among BC survivors. Adami and colleagues(Adami *et al.,* 1990) observed a slightly increased risk (10% higher) of hip fracture among a cohort of BC survivors in Sweden. However a 37% reduction in risk of hip fracture among elderly BC survivors was reported in a US study.(Lamont & Lauderdale, 2003) Kanis and colleagues(Kanis *et al.,* 1999) have reported a 20-fold greater incidence for vertebral fractures identified using a radiographic method among BC survivors who had endured soft tissue recurrences but no evidence of bone metastases. Because the radiographic method in their study is primarily used for research purposes and not for the clinical diagnosis of fractures,

the findings from Kanis *et al.*(Kanis *et al.,* 1999) are limited to subclinical fractures in a selective group of BC survivors.

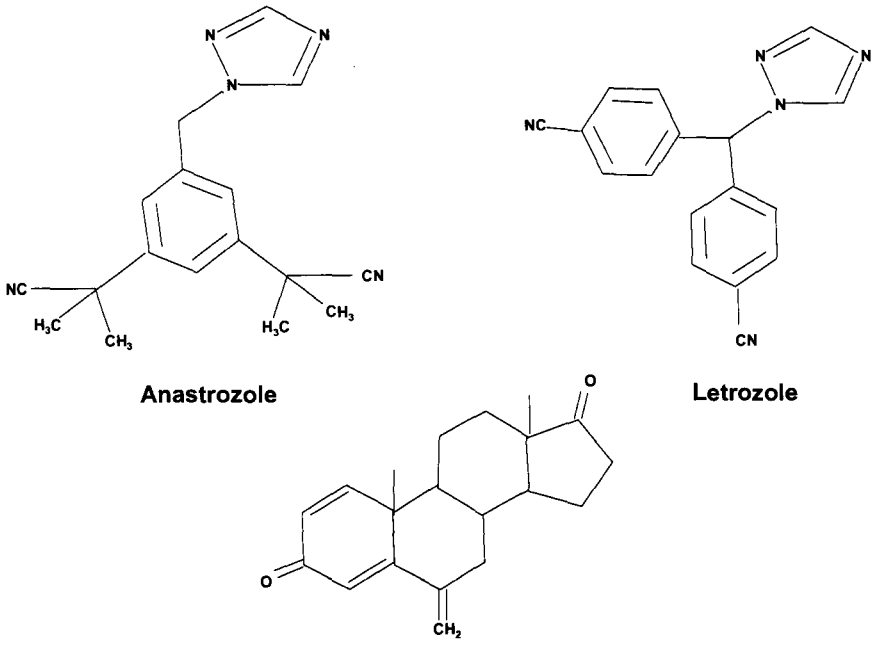
2.4.1 Aromatase Inhibitors

Aromatase ($P450_{AROM}$) is an enzyme of the cytochrome P-450 super-family and is encoded by a single gene *CYP19*, located at 15q21.2.(Evans *et al.,* 1986) It is highly expressed in the placenta and in the granulosa cells of ovarian follicles, where its expression depends on cyclical gonadotropin stimulation. Aromatase activity and $P450_{AROM}$ mRNA have also been found in several other tissues including subcutaneous fat, liver, muscle, brain, skin, bone, prostate, testis, normal breast and BC tissue.(Harada *et al.,* 1993; Mahendroo *et al.,* 1993; Means *et al.,* 1989; Miller *et al.,* 1982; Nelson & Bulun, 2001; Toda *et al.,* 1994) The level of circulating estrogen in postmenopausal women is approximately 10-20% of that in premenopausal women and achieves steady-state levels in the absence of ovarian function.(Baird & Guevara, 1969; Chakravarti *et al.,* 1976; Longcope, 1971) At menopause, mean plasma estradiol levels fall from about 110 pg per milliliter (400 pmol per litre) to low but stable levels of about 7 pg per milliliter (25 pmol per litre). Residual estrogen production after menopause is solely from the peripheral conversion of adrenal androgens in such sites as muscle and adipose tissue. Thus, peripheral aromatase activity and plasma estrogen levels correlate with BMI in postmenopausal women.(Longcope *et al.,* 1986) In postmenopausal women, aromatase activity is crucial to estrogen production within tissues and consequently to bone mass maintenance, with low serum estrogen levels sufficient to exert a restraining effect on bone turnover.(Heshmati *et al.,* 2002) Heshmati and colleagues demonstrated that reduction of the already low levels of serum estradiol to near undetectable levels in a group of

late postmenopausal women significantly increased bone resorption markers by about 15%.(Heshmati *et al.,* 2002) Similarly, Cummings *et al.*(Cummings *et al.,* 1998) reported a relationship between residual circulating estradiol and the incidence of vertebral and hip fractures in elderly women, and Stone *et al.*(Stone *et al.,* 1998) likewise described an association between serum estradiol concentrations and rates of bone loss.

Estrogen is the main hormone involved in the development and growth of breast tumors; oophorectomy was first shown to cause regression of advanced BC more than a century ago, and estrogen deprivation remains a key therapeutic approach.(Howell & Dowsett, 1997) Third generation aromatase inhibitors (AIs) are a recent development in the endocrine treatment of ER^+ BC in postmenopausal women (Figure 9).

FIGURE 9: MOLECULAR STRUCTURES OF THE THIRD GENERATION AIS*
*Adapted from Choueiri *et al.*(Choueiri TK *et al.,* 2004)



AIs markedly suppress plasma estrogen levels in postmenopausal women by inhibiting or inactivating aromatase, the enzyme responsible for the synthesis of estrogens from androgenic substrates (specifically the synthesis of estrone from the preferred substrate androstenedione and estradiol from testosterone). Due to their improved efficacy, AIs are replacing Tamoxifen as the preferred treatment for postmenopausal patients with both early and advanced estrogen-dependent BC.

There are two types of AIs, irreversible steroidal inhibitors – Exemestane (Aromasin®) - and reversible, non-steroidal inhibitors – Anastrozole (Arimidex®) and Letrozole (Femara®).(Simpson & Dowsett, 2002) Steroidal AIs are structurally similar to androstenedione and act as competitors for binding with aromatase (Figure 9). Anastrozole, Letrozole and Exemestane are administered orally. Anastrozle and Letrozole have similar pharmokinetic properties, with half-lives approximating 48 hours,(Lamb & Adkins, 1998; Wiseman & Adkins, 1998) allowing a once-daily dosing schedule. The half-life of Exemestane is 27 hours.(Lønning, 1998) The mean degree of inhibition with Anastrozole, Exemestane and Letrozole at clinical doses is greater than 97%.(Geisler *et al.,* 1998; Geisler *et al.,* 2002) Recently, subtle differences in potency between two of the third-generation inhibitors have been demonstrated. In a small, double-blind crossover trail, Letrozole was associated with greater aromatase inhibition than Anastrozole and lower plasma levels of estrone and estrone sulfate.(Geisler *et al.,* 2002) Overall, the third generation AIs appear to be very well tolerated, with a remarkably low incidence of serious short-term adverse effects, reflecting the specificity of their action.

2.4.2 Clinical Research on the Skeletal Effects of AIs

The largest trials to date, the Arimidex, Tamoxifen, Alone or in Combination (ATAC) and Breast International Group (BIG) 1-98 trials, have demonstrated that adjuvant therapy with an AI (Anastrozole or Letrozole) is associated with a significant increase in musculoskeletal disorders and fractures as compared with Tamoxifen. In the ATAC trial (n=6186), after a median follow-up of 68 months, the overall fracture rates were 11.0% and 7.7% in the Anastrozole and Tamoxifen groups, respectively (p=0.001).(Howell *et al.,* 2005) Similarly, in the BIG 1-98 trial comparing Letrozole with Tamoxifen (n=4895), after a median follow-up of 51 months, clinical fractures occurred more frequently in the AI group than in the Tamoxifen group, 8.6% versus 5.8% (p<0.001).(Breast International Group (BIG) 1-98 Collaborative Group *et al.,* 2005; Coates *et al.,* 2007) In the ATAC trial, a prospectively designed sub-protocol (n=203) assessed changes in BMD and bone biomarkers.(Eastell *et al.,* 2006) BMD was lost at the lumbar spine (median 4.1% loss) and total hip (median 3.9% loss) after two years of Anastrozole treatment. Increased bone remodeling was also observed, urinary N-telopeptide (NTX) increased 15% and bone alkaline phosphatase (ALP) increased by 20% after one year of treatment.(Eastell *et al.,* 2006) In contrast, decreased bone remodeling was observed with Tamoxifen.

In the ABCSG trial 8 and ARNO 95 trial (n=3199), postmenopausal women who had completed 2 years of adjuvant Tamoxifen were randomized to receive Anastrozole or Tamoxifen for the remainder of their adjuvant treatment.(Jakesz *et al.,* 2005) This study also noted significantly more fractures in the patients treated with Anastrozole than those on Tamoxifen (p=0.015). The NCIC CTG MA-17 trial, a placebo-controlled trial of Letrozole

after standard adjuvant Tamoxifen, found that those on Letrozole experienced more cases of patient-reported osteoporosis (5.3% *vs* 4.6% for placebo: p=0.003).(Goss *et al.,* 2003) This study also included a bone sub-protocol (n=226) to evaluate bone turnover markers and BMD in postmenopausal women randomly assigned to MA-17.(Perez *et al.,* 2006) After two years, patients receiving Letrozole had a significant decrease in total hip BMD (-3.6% *vs* -0.7%, p=0.044) and lumbar spine BMD (-5.35% *vs* -0.7%, p=0.008). Letrozole increased NTX at 6, 12, and 24 months (p=0.054, <0.001 and 0.016 respectively).(Perez *et al.,* 2006) In the Intergroup Study or IES, Exemestane was studied after 2-3 years of Tamoxifen therapy. For this study, women were randomized to complete a total of 5 years of Tamoxifen or to switch to Exemestane for the remainder of the 5 years.(Coombes *et al.,* 2007) In a bone sub-protocol for this study, after a median follow-up of 58 months, there was a trend for more clinical fractures in the Exemestane group than in the Tamoxifen group (7% *vs* 5%, p=0.003).(Coleman *et al.,* 2007) The challenge in the interpretation of these trials involves the prior use of Tamoxifen. It is likely that shortly following cessation of Tamoxifen therapy, bone turnover increases and bone loss is accelerated. Nonetheless, results of available clinical studies indicate that all three third-generation AIs affect bone turnover, BMD, and fracture risk (Table 4). These data raise concerns about the long-term effects of AIs on skeletal integrity.

TABLE 4: RANDOMIZED CONTROLLED TRIALS OF AROMATASE INHIBITORS AND THEIR SKELETAL EFFECTS

Study Name and Sample Size	Study Treatment Groups and Sample Sizes	Fracture*		Bone Sub-Protocol for BMD Sample Size	BMD in AI Group			
		Number of Subjects with a Fracture (% of Sample)	*p-value*		*Change in Spine BMD at 1 Year*[f]	*Change in Spine BMD at 2 Years*[f]	*Change in Hip BMD at 1 Year*[f]	*Change in Hip BMD at 2 Years*[f]
ATAC Trial[a] (n = 6186)	Anastrozole (n=3092)	340 (11.0%)	p<0.001	94	-2.6%	-4.0%	-1.7%	-3.2%
	vs							
	Tamoxifen (n=3094)	237 (7.7%)		109	+1.2%	+1.9%	+0.8%	+1.2%
BIG 1-98[b] (n = 4895)	Letrozole (n=2448)	211 (8.6%)	p<0.001	N/A	N/A	N/A	N/A	N/A
	vs							
	Tamoxifen (n=2447)	141 (5.8%)						
IES[c] (n = 4658)	Exemestane following Tamoxifen (n=2320)	162 (7.0%)	p=0.003	101	-3.6%	-4.0%	N/A	N/A
	vs							
	Tamoxifen (n=2338)	115 (4.9%)		105	NSC	NSC		
MA-17[d] (n = 5149)	Letrozole following Tamoxifen (n=2572)	137 (5.3%)	N/A	122	-3.3%	-5.4%	-1.4%	-3.6%
	vs							
	Placebo following Tamoxifen (n=2577)	119 (4.6%)		104	-2.5%	-0.7%	-2.4%	-0.7%
ABCSG-8/ ARNO-95[e] (n = 3199)	Anastrozole following Tamoxifen (n=1602)	34 (2.1%)	p=0.015	N/A	N/A	N/A	N/A	N/A
	vs							
	Tamoxifen (n=1597)	16 (1.0%)						

[a](Baum *et al.* 2002; Howell *et al.* 2005; Eastell *et al.* 2006), [b](Breast International Group (BIG) 1-98 Collaborative Group *et al.* 2005; Coates *et al.* 2007), [c](Coleman *et al.* 2007; Coombes *et al.* 2007), [d](Goss *et al.* 2005; Perez *et al.* 2006;), [e](Jakesz R *et al.* 2005); *Fracture incidence measured in total cohort for each study. [f]BMD Change p<0.05 relative to baseline.

2.5 SUMMARY OF THE LITERATURE REVIEW

The cessation of ovarian function with the onset of menopause is important in the pathogenesis of bone fragility. Without sufficient estrogen levels, osteoclast activity is increased and survival is prolonged. This occurs because estrogens normally interfere with transcriptional regulation of a number of cytokines and cytokine-related factors that influence the differentiation of osteoclast progenitors and osteoclast survival. Concurrently, osteoblastic activity is reduced because of the loss of stimulatory effects of estrogens on growth factors important for bone formation. Therefore, estrogen deficiency results in a net loss of bone mass. The increased numbers of remodeling sites and deeper resorption lacunae produce increased porosity and loss of connectivity in cortical and trabecular bone.

DXA measurements of BMD are the accepted standard for diagnosing changes in bone mass. These measurements provide an estimate of the relative risk of fracture, but their utility in individual patient risk assessment is limited. The limitations of DXA measurements have prompted research for more reliable and responsive tools to measure bone material quality and macro-architecture. pQCT technology permits the vBMD of cortical and trabecular bone to be assessed as well as allowing for a noninvasive evaluation of the geometric properties at appendicular skeletal sites including the radius and tibia. This presents a means of assessing bone strength and fracture risk, beyond the scope of current DXA determinations. pQCT also provides a means of examining the response of mature bone to various treatment regimens which can be detected as changes in geometry, and increased, decreased, or redistributed mineral mass, which ultimately results in variations in bone strength and risk of fracture.

In postmenopausal women with a history of BC, cancer-associated bone morbidity is a major public health concern due to antineoplastic treatments that reduce residual circulating estrogen levels dramatically. A lack of awareness concerning the potential long-term adverse effects of AIs on skeletal health may lead to chronic pain, disability, and decreased quality of life. To improve therapy, it is important to carefully evaluate the pathophysiology of the effects of AIs on bone health, including the effects of these medications on cortical and trabecular bone.

Chapter 3

MATERIALS AND METHODS

3.1 Study Participants

For all the sub-studies which comprise this thesis, the presence of diseases or the use of medications known to affect bone metabolism were exclusion criteria for participants. The only exception was during the 24-month follow-up study, 1 subject was prescribed a bisphosphonate during the course of the follow-up period and this is noted in the results for that study. Postmenopausal status was determined by self-report and natural menopause was defined as the age at which menses ceased for a period of twelve months. For women who had undergone surgical hysterectomy and/or ovariectomy, the age at surgery was recorded. All subjects gave informed, written consent prior to participating. Each study was approved by the Hamilton Health Sciences Research Ethics Board.

3.1.1 pQCT Measurement Reproducibility: Study Participants

Healthy females, over 18 years of age, were recruited from staff, students, and outpatients at McMaster University Medical Centre (Hamilton, Ontario). Twenty-nine women were recruited for this study; fourteen were premenopausal and fifteen were postmenopausal. Fifteen of the volunteers (seven premenopausal and eight postmenopausal) participated in the upper limb study, and fifteen volunteers (also seven premenopausal and eight postmenopausal) participated in the lower limb study.

3.1.2 Osteoporosis Knowledge, pQCT, and DXA: Study Participants

Healthy postmenopausal females were recruited from staff and outpatients at McMaster University Medical Centre. Postmenopausal breast cancer patients were recruited from the Juravinski Cancer Centre (Hamilton, Ontario). All cancer patients had completed treatment

for Stage I or II breast carcinoma and were prescribed the Aromatase Inhibitor, Anastrozole. Subsequent to their agreement to enter the study, all breast cancer subjects underwent a DXA BMD scan, serum analysis, and consulted with a rheumatologist. For each sub-group, exclusion criteria were prior diagnosis of osteoporosis and other metabolic bone diseases. Fifty-eight women were recruited for this study, of these women, 27 had been diagnosed with breast cancer and 31 were healthy control subjects.

3.1.3 Anastrozole Follow-up pQCT Scans: Study Participants

Eight postmenopausal breast cancer patients were recruited from the Juravinski Cancer Centre (Hamilton, Ontario). All cancer patients had completed treatment for Stage I or II breast cancer and were prescribed the Aromatase Inhibitor, Anastrozole. Follow-up pQCT scans were performed 1 yr (1.0 ± 0.1 yr) from the date of the baseline scan and a third set of scans was performed 2 years (1.9 ± 0.1 yr) after the baseline measurement, in a sub-set of 8 and 5 breast cancer subjects respectively. The same patient positioning procedure (described in section *3.3.1*) used in the initial scan was applied in the follow-up scans to ensure the consistency of image-slice location and reproducibility of patient positioning. The design of this follow-up study utilized the postmenopausal breast cancer subject as a self-control model, which removed the influence of confounding variables, such as genetic, nutritional and hormonal factors.

3.1.4 Medical History Assessment

All study subjects completed a questionnaire to document age, age at menopause, race/ethnicity, height, body weight, and a general health/medical history profile. The body

mass index (BMI) was calculated as weight/height^2 for each subject. Additional information on smoking habit, time spent exercising, family history of osteoporosis, use of medications, fracture history, and dietary intake were also assessed. The completed questionnaire was reviewed and confirmed with an interview at the time of pQCT measurement. Study participants had the option to not respond to questions which they felt uncomfortable or unwilling to answer; any corresponding variation in sample size is noted in the results.

3.2 Osteoporosis Knowledge Questionnaire

Participant's knowledge of osteoporosis was assessed using the Facts on Osteoporosis Quiz (FOOQ) developed by Ailinger *et al.*(Ailinger *et al.,* 1998) The FOOQ is a validated, psychometrically sound instrument for collecting this data.(Ailinger *et al.,* 1998; Ailinger *et al.,* 2003; Ailinger & Emerson, 1998) The FOOQ consists of 20 true or false questions related to risk factors, self-care factors, disease manifestations, preventative behavior, and treatment associated with osteoporosis. Internal consistency and reliability for the FOOQ, based on Cronbach's alpha coefficient, was measured to be 0.84.(Ailinger *et al.,* 2003) With the FOOQ, in addition to the '*true*' and '*false*' responses, there is also a '*don't know*' response option. This third option allows respondents a choice to reduce guessing and to discriminate between a specific lack of knowledge (*don't know* response) and misinformation (incorrect response). Participant responses were initially examined to note whether they were due to lack of information or misinformation. Subsequently, in calculating the total raw score for the instrument, each item was then assigned a score of 1 for a correct answer, and 0 if the answer was incorrect or a *don't know.* A total possible score on the FOOQ could range from 0 to 20, with higher scores indicating more knowledge of osteoporosis. The questionnaire

took approximately 5 minutes for each participant to complete. The authors permission letter for the use of the FOOQ, a copy of the FOOQ, and the answer key are displayed in Appendices G through I.

3.3 pQCT Measurements

pQCT measurements were taken at two different sites of the radius and three sites of the tibia using a Stratec XCT-2000 pQCT scanner (manufactured by Stratec Medizintechnik, Pforzheim, Germany, and distributed in North America by Orthometrix Inc, White Plains New York, USA). The device is a translate-rotate, small-bore CT scanner that acquires a trans-axial image. The x-ray source (58 kV, 180 μA) is collimated to produce a narrow fan beam with an effective width of 2.3 mm. The total effective radiation dose associated with each examination is less than 2 μSv. Images were acquired with an in-plane voxel dimension of 0.2 mm (0.008 mm^3). To ensure machine stability, the pQCT device was assessed daily based on a Quality Control Phantom, supplied by the manufacturer, which includes soft tissue equivalent material.

3.3.1 Scanning Procedure

For each participant, the non-dominant arm or leg was selected for measurement on the basis of whether the patient was right- or left-handed. Exceptions were made when the non-dominant arm or leg had been fractured within the last 10 years. The subjects were seated on a stationary chair, adjusted to the appropriate height, and offered the use of a foot stool and cushion to optimize comfort. For the radius scans, the length of the bone from the humero-radial joint cleft to the styloid process was measured. For the tibia scans, the length of the

bone from the distal end of the medial malleolus to the medial knee joint cleft was measured. A radial or tibial adjustable clamp was used to support the limb and to limit motion during the scans. Care was taken to ensure that the limb being scanned was well supported and centered appropriately in the imaging field.

The scanner was positioned on the distal radius or distal tibia and a coronal computed radiograph (scout view) was carried out by the operator to manually locate a reference line on the distal end of either the radius or the tibia. The measurement sites were located proximal to this reference line by a distance corresponding to 4% (*Distal Radius*) and 20% (*Diaphyseal Radius*) of the radius length, and 4% (*Distal Tibia*) and 38% (*Diaphyseal Tibia*) of the tibia length. For the measurement of muscle, fat, and bone cross-sectional area the site utilized was at 66% of the length of the tibia (*Muscle, Fat, and Bone Cross-Sectional Area*), where the largest calf diameter is typically located. For these variables, the device was manually positioned and the scan was performed without a scout scan. The reproducibility for each site was determined by performing the measurements twice on each subject. The subjects were repositioned between the measurements and a new scout view was performed for the second measurement. Each scan required approximately 90 seconds, with some variability depending on the cross-sectional size of the forearm or lower leg. The same operator acquired images of the radius and tibia for each subject. Appendix B depicts the anatomical measurement sites of the radius and the tibia.

3.3.2 Measurement Parameters

Image analysis and the selection of threshold values were performed using the manufacturer's

software, version 5.40. The following parameters were determined at the selected bone sites: (a) the mass of mineralized tissue in the cross-section - bone mineral content (BMC in mg); (b) the size of the cross-sectional area of the bone - area (in mm^2); and (c) volumetric bone mineral density (vBMD in mg/cm^3). Parameters included total vBMD (TOT_DEN), trabecular vBMD (TRAB_DEN), cortical vBMD (CRT_DEN), total content (TOT_CNT), trabecular content (TRAB_CNT), cortical content (CRT_CNT), total cross-sectional area (TOT_A), trabecular cross-sectional area (TRAB_A), cortical cross-sectional area (CRT_A), cortical thickness (CRT_THK), endocortical circumference (ENDO_CIR), periosteal circumference (PERI_CIR), polar moment of inertia (PMI), polar moment of resistance (PMR), and polar stress strain index (SSI).

To define the outer boundary of the bone and to distinguish trabecular bone from cortical bone, the pQCT system uses a contour algorithm that detects the periosteal surface of the bone based on a threshold of 280 mg/cm^3. After detecting the outer bone contour, there is concentric peeling of the outer 55% of voxels until a central area covering 45% of the total bone cross-sectional area remains. From this central area, TRAB_DEN is determined. The actual relative cross-sectional area of the trabecular compartment is considerably larger than 45%; however, the resolution of the pQCT system is not sufficient to trace the border between trabecular and cortical bone. Therefore, this geometric definition of trabecular bone includes some margin of safety to exclude cortical bone from the trabecular region of interest. In the cortical compartment, many voxels are only partially occupied by cortical bone. At a threshold of 710 mg/cm^3, the number of such voxels that are included in the analysis is equivalent to the number excluded. CRT_DEN was measured as the mass of the mineral

above this threshold divided by the volume occupied which allows the error due to the partial volume effect to be minimized. In this study, cortical bone parameters were only determined at the diaphyseal sites of the radius and tibia.

For the analysis of muscle cross sectional area, the region of interest (ROI) was defined to include the entire matrix (skin, subcutaneous tissue, muscle and bone). Within this ROI, the total area of the muscle was determined with the threshold set at 40 mg/cm^3 and the total bone was assessed at the threshold of 710 mg/cm^3. Next, the total area of skin and subcutaneous fat was identified using a threshold of -100 mg/cm^3. Subsequently, the total bone area and total areas of skin and subcutaneous fat were deducted from the ROI to yield the total muscle area. No additional volume was removed from inside the muscle with this approach.

3.4 DXA Measurements

Each breast cancer subject underwent a Hologic Delphi^{TM} DXA (Hologic Inc., Bedford, MA, USA) scan of their posterior-anterior spine, total hip and total body. All subjects were referred to the same site (Hamilton, Ontario) for this testing. Standard DXA protocols were used for participant positioning and analysis of DXA images by one trained technician. The Hologic device was calibrated daily with a standard phantom; the *in vitro* coefficient of variation for the lumbar spine quality control phantom during the duration of the study was 0.37%. The measurement parameters included; Bone Mineral Content (BMC in g), Bone Mineral Density (BMD in g/cm^2), Fat Mass (FM in g and %), Lean Body Mass (LBM in g), and Total Mass (TM in g).

3.4.1 Serum Mineral, Hormone, and Vitamin Levels

At the time of the DXA scan, all breast cancer subjects underwent routine blood work. The results for serum levels of calcium (nmol/L), inorganic phosphorus (nmol/L), intact parathyroid hormone (pmol/L) and vitamin D (25-OH vitamin D) (nmol/L) were recorded.

3.5 Data Analysis

Quantitative analysis was performed for the cohort as a whole and statistical analyses were also conducted to compare the healthy and breast cancer subject subgroups. Data analysis included the following; (*i*) descriptive statistics indicating the number of subjects (*n*), mean, standard deviation (SD), and standard error of the mean (SEM) values were calculated for all anthropometric characteristics of the study subjects and all measured variables of bone density, mass, and geometry; (*ii*) the precision error for all pQCT measurement sites was calculated as the root-mean-square coefficients of variation (*rms*CV) of duplicate measurements using the method proposed by Gluer *et al.*(Gluer *et al.*, 1995); (*iii*) Anderson Darling tests for normality, including 95% confidence intervals, were conducted on all the data sets to ensure normal distribution for parametric data analysis; (*iv*) students t-tests were used for inter-group comparisons; (*v*) the strength of the linear association between the measurement sites was calculated using Pearson Product Moment Correlation Coefficients (*r*); (*vi*) any data patterns associated with the independent variables were detected using linear regression analysis, in all cases the R^2 value (%) and the equation of the linear regression line is reported; (*vii*) the ANOVA linear mixed effects model approach was utilized to evaluate the subgroup differences at baseline, first, and second follow-up visits; (*viii*) all calculations were conducted on the original data in accordance with the valid application of the test of

significance; differences were considered significant at $p<0.05$ and $p<0.01$. The statistical results have been calculated using the statistical package, Minitab (release 13.1).

Chapter 4

RESULTS

4.1 Reproducibility of pQCT Measurements at the Radius and Tibia in Healthy Pre- and Postmenopausal Women

The first sub-study of this thesis involved an investigation into the reproducibility of pQCT measurements at five anatomical sites of the radius and tibia. Twenty-nine subjects were recruited to participate in this study; 14 premenopausal women and 15 postmenopausal women had repeated pQCT measurements taken of their radius or tibia. The anthropometric characteristics of the subjects are provided in Table 5. The mean (SD) age of the premenopausal participants was 27.6 (7.5) years and for postmenopausal participants was 55.3 (8.4) years. There were no statistically significant differences between the two populations with respect to height, mass, or body mass index.

TABLE 5: ANTHROPOMETRIC DATA OF THE STUDY PARTICIPANTS

Variable[a]	Premenopausal (n = 14)	Postmenopausal (n = 15)	All Subjects (n = 29)
Age (years)	28 (8)	55 (8)	42 (16)
Height (m)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)
Mass (kg)	64.0 (11.6)	68.7 (12.7)	66.4 (12.2)
Body Mass Index (kg/m^2)	22.6 (3.1)	25.0 (5.2)	23.8 (4.4)

[a]Data expressed as the mean (SD)

4.1.1 Distal Radius and Tibia

Trabecular bone parameters were analyzed at the distal sites and cortical bone parameters were analyzed at the proximal sites in order to evaluate reproducibility at the radius and tibia with the XCT-2000 pQCT scanner. As a consequence of insufficient spatial resolution at the bone edges (partial volume effect), cortical bone parameters can be underestimated at distal skeletal sites. The literature indicates three variant cut-off points for accurate cortical bone

measurements, suggesting that this effect is greatest for cortices thinner than either 2.5 mm,(Hangartner & Gilsanz, 1996) 4 mm,(Augat *et al.,* 1998c) or 1.2 mm.(Prevrhal *et al.,* 1999) In the subjects of this sub-study, the mean (SD) cortical thickness was 0.71 (0.04) mm at the distal radius, 0.44 (0.04) mm at the distal tibia, 2.48 (0.7) mm at the diaphyseal radius, and 4.88 (0.1) mm at the diaphyseal tibia. Tables 6 and 7 list the pQCT variables for pre- and postmenopausal women at the distal radius and tibia respectively.

TABLE 6: REPRODUCIBILITY OF BONE VARIABLES AT THE 4% DISTAL RADIUS

Variable[a]	Pre-Menopausal (n = 7)	Post-Menopausal (n = 8)	All Subjects (n = 15)	*rms*SD (n = 15)	*rms*CV (%) (n = 15)
Density (mg/cm^3)					
TOT_DEN	365 (11.6)	368 (25.2)	365 (14.1)	3.8	5.1
TRAB_DEN	201 (9.5)	193 (12.9)	197 (7.9)	0.7	2.1
Mass (mg)					
TOT_CNT	117 (5.0)	105 (4.0)	111 (3.4)	0.4	2.2
TRAB_CNT	29 (1.8)	25 (1.5)	27 (1.2)	0.3	6.7
Area (mm^2)					
TOT_A	321 (11.1)	295 (18.5)	308 (11.1)	3.9	6.5

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; TRAB_DEN, trabecular density; TOT_CNT, total content; TRAB_CNT, trabecular content; TOT_A, total area

TABLE 7: REPRODUCIBILITY OF BONE VARIABLES AT THE 4% DISTAL TIBIA

Variable[a]	Pre-Menopausal (n = 7)	Post-Menopausal (n = 8)	All Subjects (n = 15)	*rms*SD (n = 15)	*rms*CV (%) (n = 15)
Density (mg/cm^3)					
TOT_DEN	317 (10.3)	267 (12.6)*	290 (10.4)	0.4	1.5
TRAB_DEN	241 (8.3)	203 (7.6)**	221 (7.4)	0.5	1.6
Mass (mg)					
TOT_CNT	317 (20.0)	273 (16.6)	294 (13.8)	0.8	1.6
TRAB_CNT	109 (7.0)	94.0 (5.7)	101 (4.7)	0.6	4.3
Area (mm^2)					
TOT_A	1001 (54.0)	1024 (42.5)	1013 (32.7)	4.0	2.8

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; TRAB_DEN, trabecular density; TOT_CNT, total content; TRAB_CNT, trabecular content; TOT_A, total area; *$p < 0.05$, **$p<0.01$ for 2 sample t-test

Also given in each table, where applicable, is the statistical significance of the difference between pre- and postmenopausal women. Mean values for TRAB_DEN and TOT_DEN at the distal radius were 201 mg/cm^3 and 365 mg/cm^3 respectively in premenopausal women. Results were similar in postmenopausal women, measuring 193 mg/cm^3 and 368 mg/cm^3. None of the variables were statistically significantly lower in the radii of postmenopausal women. However, at the distal tibia, results for TRAB_DEN and TOT_DEN were significantly ($p<0.01$ and $p<0.05$) lower in postmenopausal women at 203 mg/cm^3 and 267 mg/cm^3, as compared to premenopausal women with mean measurements of 241 mg/cm^3 and 317 mg/cm^3. Interestingly, the differences in TOT_A between the pre- and postmenopausal women at both the radius and tibia were not statistically significant.

At the distal radius, TRAB_DEN measurements had the best reproducibility (*rms*CV of 2.1%), while for TOT_DEN *rms*CV was higher, at 5.1%. For the mass and geometry parameters, the *rms*CVs were higher with the exception of TOT_CNT (2.2%). At the distal tibia, reproducibility was markedly better than that observed at the distal radius. The *rms*CV for TRAB_DEN was 1.6% and for TOT_DEN was 1.5%.

4.1.2 Diaphyseal Radius and Tibia

Tables 8 and 9 present the results for bone and geometric variables at the diaphyseal sites of the radius and tibia respectively.

TABLE 8: REPRODUCIBILITY OF BONE VARIABLES AT THE 20% DIAPHYSEAL RADIUS

Variable[a]	Pre-Menopausal (n = 7)	Post-Menopausal (n = 8)	All Subjects (n = 15)	*rms*SD (n = 15)	*rms*CV (%) (n = 15)
Density (mg/cm^3)					
TOT_DEN	890 (24.5)	916 (34.9)	899 (22.6)	4.4	3.3
CRT_DEN	1253 (6.8)	1266 (10.4)	1259 (6.7)	1.2	0.5
Mass (mg)					
TOT_CNT	97 (4.2)	94 (2.3)	95 (2.3)	0.2	1.4
CRT_CNT	91 (4.1)	88 (2.3)	89 (2.2)	0.1	0.6
Area (mm^2)					
TOT_A	110 (6.1)	103 (4.4)	107 (3.8)	0.7	3.8
CRT_A	73 (3.5)	70 (1.7)	71 (1.8)	0.1	0.9
Geometry					
CRT_THK *(mm)*	2.5 (0.1)	2.5 (0.1)	2.5 (0.7)	0.01	3.1
PERI_CIR *(mm)*	37 (1.0)	36 (0.8)	37 (0.7)	0.1	5.5
ENDO_CIR *(mm)*	22 (1.0)	20 (1.3)	21 (0.9)	0.2	1.9
PMI *(mm^3)*	1698 (189)	1494 (91)	1593 (101)	6.4	2.1
PMR *(mm^3)*	229 (16)	198 (7)	213 (9)	0.8	2.0
SSI *(mm^3)*	138 (16)	208 (7)	222 (9)	0.7	1.7

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; CRT_DEN, cortical density; TOT_CNT, total content; CRT_CNT, cortical content; TOT_A, total area; CRT-A, cortical area; CRT-THK, cortical thickness; ENDO_CIR, endosteal circumference; PERI_CIR, periosteal circumference; PMI; polar moment of inertia; PMR, polar moment of resistance; SSI, stress-strain index

TABLE 9: REPRODUCIBILITY OF BONE VARIABLES AT THE 38% DIAPHYSEAL TIBIA

Variable[a]	Pre-Menopausal ($n = 7$)	Post-Menopausal ($n = 8$)	All Subjects ($n = 15$)	*rms*SD ($n = 15$)	*rms*CV (%) ($n = 15$)
Density (mg/cm^3)					
TOT_DEN	896 (15.1)	884 (21.3)	882 (17.1)	6.7	5.7
CRT_DEN	1205 (8.4)	1204 (12.2)	1205 (7.4)	0.7	0.3
Mass (mg)					
TOT_CNT	341 (21.3)	309 (11.9)	324 (12.2)	0.7	1.3
CRT_CNT	324 (19.9)	295 (10.4)	308 (11.1)	0.3	0.5
Area (mm^2)					
TOT_A	381 (22.7)	353 (17.6)	369 (14.4)	2.9	4.9
CRT_A	269 (17.5)	245 (9.3)	256 (9.7)	0.3	0.6
Geometry					
CRT_THK *(mm)*	5.0 (0.2)	4.8 (0.1)	4.9 (0.1)	0.03	4.6
PERI_CIR *(mm)*	69 (2.0)	66 (1.7)	68 (1.3)	0.3	2.5
ENDO_CIR *(mm)*	37 (1.3)	36 (2.0)	37 (1.4)	0.5	7.5
PMI *(mm^3)*	22480 (3265)	18120 (1539)	20146 (1763)	45.8	1.1
PMR *(mm^3)*	1486 (149)	1308 (86)	1391 (84)	2.9	1.1
SSI *(mm^3)*	1479 (145)	1310 (83)	1389 (81)	3.7	1.2

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; CRT_DEN, cortical density; TOT_CNT, total content; CRT_CNT, cortical content; TOT_A, total area; CRT-A, cortical area; CRT-THK, cortical thickness; ENDO_CIR, endosteal circumference; PERI_CIR, periosteal circumference; PMI; polar moment of inertia; PMR, polar moment of resistance; SSI, stress-strain index

Mean values for CRT_DEN at the diaphyseal radius in premenopausal and postmenopausal women were 1253 mg/cm^3 and 1266 mg/cm^3 respectively, and at the diaphyseal tibia these data were very similar at 1205 mg/cm^3 and 1204 mg/cm^3 respectively. There were no statistically significant differences between pre- and postmenopausal women at the diaphyseal radius or tibia for any of the measured density or mass variables. Likewise, the PMI, PMR and SSI, which take into account both density and geometry data derived from pQCT, were not significantly different at the diaphyseal radius or tibia between pre- and postmenopausal women.

At the diaphyseal radius site, where the cortex is thicker, the *rms*CV for CRT_DEN was 0.5%. The other cortical bone parameters here were almost as precise with CRT_CNT at 0.6% and CRT_A at 0.9%. At the diaphyseal tibia, the precision of the cortical measurements were similar to that at the radius with *rms*CV of 0.3% for CRT_DEN, 0.5% for CRT_CNT and 0.6% for CRT_A. When examining the correlations between the TOT_DEN values at all four measurement sites (Table 10), the correlation between TOT_DEN at the 4% radius and the 20% radius was the greatest (r=0.64, p=0.01).

TABLE 10: PEARSON CORRELATION COEFFICIENTS AND P-VALUES FOR TOT_DEN

Variable[a]	TOT_DEN (4% radius)	TOT_DEN (4% tibia)	TOT_DEN (20% radius)
TOT_DEN (4% tibia)	-0.19 p=0.49		
TOT_DEN (20% radius)	0.64 p= 0.01*	-0.23 p=0.41	
TOT_DEN (38% tibia)	0.15 p=0.59	0.42 p=0.12	0.34 p=0.21

[a]Cell contents; Pearson correlation coefficient, p-value; *significant at p<0.05

4.1.3 Muscle, Subcutaneous Fat, and Bone Cross-Sectional Area

Positioning at the 66% site was occasionally difficult due to the size of the leg aperture (14 cm diameter) in relation to the diameter of a subject's calf. One study patient was unable to be scanned at the 66% tibia site due to an inability to properly fit the leg into the scanning aperture. However, in this population, patient positioning difficulties were not experienced at the 38% tibia site. Table 11 lists the results for muscle, fat, and bone variables in pre- and post-menopausal women assessed at the 66% tibia site. There were no statistically significant differences between pre- and postmenopausal women for these parameters at this site. The reproducibility for all parameters at the 66% tibia site was good, with total *rms*CV values between 0.6 and 0.7%.

TABLE 11: REPRODUCIBILITY OF CROSS-SECTIONAL MUSCLE, SUBCUTANEOUS FAT, AND BONE AREA AT THE 66% TIBIA

Variable[a]	Pre-Menopausal ($n = 6$)	Post-Menopausal ($n = 8$)	All Subjects ($n = 14$)	*rms*SD ($n = 14$)	*rms*CV (%) ($n = 14$)
Area (mm^2)					
Total muscle	5680 (228)	5904 (208)	5808 (151)	6.3	0.6
Total fat	4523 (440)	4730 (195)	4641 (211)	7.1	0.7
Total bone[b]	332 (18.1)	315 (15.5)	323 (11.5)	0.5	0.7

[a]Data expressed as the mean (SEM); [b]includes total tibia and fibula

4.2 An Assessment of Osteoporosis Knowledge in Postmenopausal Women: A Focus on Women with a History of Breast Carcinoma

An investigation into the level of patient knowledge regarding osteoporosis – diagnosis, preventative measures, and treatment options – was undertaken to identify any differences between healthy postmenopausal women and women with a history of breast carcinoma. The mean (SD) age of all participants was 60 (8) years (range 46 to 81 years) and in the subgroups, 58 (7) for healthy postmenopausal women and 62 (9) for breast cancer subjects. There were no significant differences between the two populations with respect to age, mass, height, BMI, or number of biological children.

The mean age (SD) at menopause was similar for both subject groups; 49 (7) for the healthy postmenopausal women and 48 (6) for the breast cancer subjects. The mean years since menopause was higher in the breast cancer group at 14 (9) versus 9 (9), but this difference was not statistically significant. One subject in each subgroup did not submit her age at menopause, thus, this data is not included in the analysis and the sample size for this parameter is n=26 for the breast cancer subjects and n=30 for the healthy control subjects. Twenty-three participants had undergone surgical hysterectomy (n=8 controls and n=15 breast cancer subjects) and fourteen (n=7 for each group) had undergone surgical ovariectomy; the mean ages for these surgeries were 45 years and 44 years respectively. The mean score of the FOOQ was 15 (3) for the healthy control group and significantly lower ($p<0.01$) at 13 (3) for the breast cancer subjects. The anthropometric characteristics of the study participants are given in Table 12; separate analysis is provided for each subject group.

TABLE 12: ANTHROPOMETRIC DATA OF THE STUDY PARTICIPANTS

Variable[a]	All Subjects (n = 58)	Healthy Postmenopausal Subjects (n = 31)	Breast Cancer Subjects (n = 27)
Age (years)	60 (8)	58 (7)	62 (9)
Age at Menopause[b] (years)	49 (6)	49 (7)	48 (6)
Years Since Menopause[b] (years)	11 (9)	9 (9)	14 (9)
Height (m)	1.63 (0.06)	1.62 (0.06)	1.64 (0.07)
Mass (kg)	71.4 (13.6)	69.3 (12.1)	73.8 (15.0)
Body Mass Index (kg/m^2)	27.0 (5.4)	26.5 (5.1)	27.6 (5.7)
Number of Biological Children	2 (0.1)	2 (0.2)	2 (0.2)
Total mean score on the FOOQ	14 (3)	15 (3)	13 (3)**

[a]Data expressed as the mean (SD);[b]Data set consists of n=26 breast cancer patients, n=30 healthy postmenopausal women, and n=56 total participants;**$p<0.01$ for 2 sample t-test

4.2.1 Participant Characteristics

Ninety-five percent of the study subjects were white/caucasian, there was one participant each of Hispanic, Asian, and African-American ethnicity. Seventy-nine percent of participants stated that they had never fractured a bone as an adult. For those that had experienced a fracture, the sites involved were those typically associated with traumatic fractures such as the clavicle, phalanges, fibula, and coccyx. Three participants had fractures that may have been due to bone fragility; two of the radius and one of the ankle. Approximately half of all participants (53%) stated that they had been informed about osteoporosis, 58% of healthy controls and 10% fewer of the breast cancer subjects (48%) stated that they had spoken to a medical professional about osteoporosis. Of those that had spoken to a medical professional about osteoporosis, 83% had spoken to their family doctor, 6% to their rheumatologist, and the remaining subjects had spoken to either a bone mineral density measurement technician (6%) or to a previous study coordinator (3%) (unrelated to this study). With respect to a

family history of osteopenia or osteoporosis, participants were asked to indicate if anyone in their immediate family had ever shown signs of bone loss. Fifty percent indicated that in their recollection a member of their family had been diagnosed with osteoporosis or had shown symptoms of the disease. Again, approximately 10% fewer breast cancer subjects (55% healthy controls in comparison with 44% breast cancer subjects) reported a family history of osteoporosis. Of all these subjects, 40% indicated that their mother had either been diagnosed with osteoporosis, developed a stoop with age (loss of height) or had fractured her hip or wrist, 10% indicated that their father had developed osteoporosis or exhibited a loss of height and 9% indicated that either their sister or brother had incurred a loss of height. With respect to cigarette smoking, 55% of all participants had never been smokers, 9% were currently smokers, and 36% had been smokers in the past but had since quit. Fifty-nine percent of participants regularly consumed coffee on a daily basis (mean of 2 cups per day) and 34% regularly consumed some form of alcohol (mean of 2 servings per day) on a daily basis. Calcium, vitamin D, and multivitamin supplements were assessed separately at any dose as long as the supplements were taken regularly. Seventy-nine percent of participants regularly took calcium supplements and 67% took separate vitamin D supplements with 52% taking multivitamin supplements.

With respect to physical activity, 55% reported that they did participate in some form of regular physical activity (one or more times per week for 15 minutes or longer). When investigating the subgroups separately, this proportion was much higher for the healthy controls (71%) than for the breast cancer subjects (37%). The breakdown for the average number of hours spent doing strenuous sports (including jogging, bicycling, tennis,

racquetball, aerobics, *etc.*), vigorous weight-bearing activities (including shoveling, weight lifting, or equivalent manual labor), and moderate activity (including housework, brisk walking, golfing, gardening, *etc.*) is shown graphically in Figures 10 to 12. It is important to note that it was frequently difficult for the study participants to differentiate which activities are weight-bearing and which are not. Overall, the breast cancer subjects participated in less strenuous sports and weight-bearing activities than the postmenopausal controls. Of the respondents, 7% considered themselves to be a lot less active than their peers, 14% rated themselves as being somewhat less active, 36% considered themselves about the same, 24% deemed themselves somewhat more active, and 17% regarded themselves as being a lot more active. Regrettably, the response to this question could not be verified by an independent examination. An analysis of each measured medical history variable with respect to the two subgroups is listed in Table 13.

TABLE 13: MEDICAL HISTORY OF THE STUDY PARTICIPANTS

Variable[a]	All Subjects (n = 58)	Healthy Postmenopausal Subjects (n = 31)	Breast Cancer Subjects (n = 27)
Ethnic Background			
Caucasian	55 (95%)	28 (90%)	27 (100%)
Hispanic	1 (2%)	1 (3%)	0
African-American	1 (2%)	1 (3%)	0
Asian	1 (2%)	1 (3%)	0
Right Hand Dominance	50 (86%)	25 (81%)	25 (93%)
Hysterectomy	23 (40%)	8 (26%)	15 (56%)
Ovariectomy	14 (24%)	7 (23%)	7 (26%)
Has anyone ever spoken to you about osteoporosis?	31 (53%)	18 (58%)	13 (48%)
As an adult, have you ever broken a bone?	12 (21%)	6 (19%)	6 (22%)
Family History of Osetopenia or Osteoporosis	29 (50%)	17 (55%)	12 (44%)
Smoking History			
Never	32 (55%)	18 (58%)	14 (52%)
Current	5 (9%)	1 (3%)	4 (15%)
Past	21 (36%)	12 (39%)	9 (33%)
Regular Coffee Consumption	34 (59%)	16 (52%)	18 (67%)
Regular Alcohol consumption	20 (34%)	11 (35%)	9 (33%)
Do you take:			
Calcium supplementation (any dose)	46 (79%)	19 (61%)	27 (100%)
Vitamin D supplementation (any dose)	39 (67%)	13 (42%)	26 (96%)
Multivitamin supplementation	30 (52%)	18 (58%)	12 (44%)
Do you currently participate in regular physical activity?	32 (55%)	22(71%)	10 (37%)
Rate your overall physical activity compared with your peers:[b]			
A Lot Less	4 (7%)	1 (3%)	3 (11%)
Somewhat Less	8 (14%)	3 (10%)	5 (19%)
About the Same	21 (36%)	8 (26%)	13 (48%)
Somewhat More	14 (24%)	10 (32%)	4 (15%)
A Lot More	10 (17%)	8 (26%)	2 (7%)

[a]Data expressed as subset of n and (% with 'yes' response); [b]Data set consists of n=30 healthy postmenopausal women, and n=57 total participants

FIGURE 10: THE NUMBER OF HOURS PER WEEK INVOLVED IN STRENUOUS SPORTS

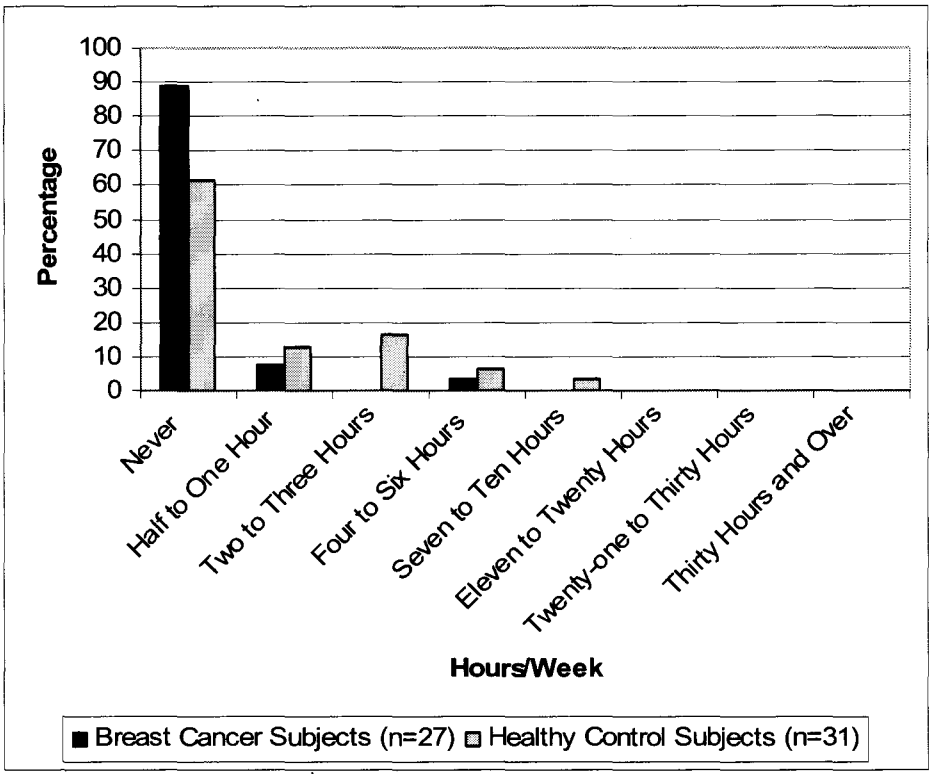


FIGURE 11: THE NUMBER OF HOURS PER WEEK INVOLVED IN VIGOROUS WORK

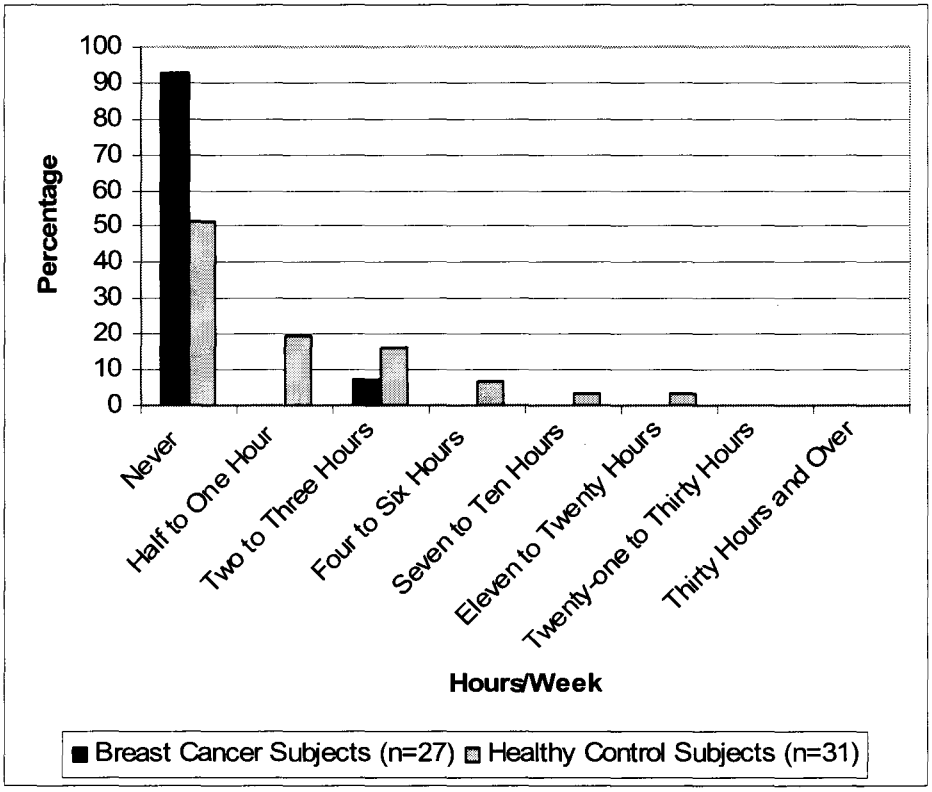


FIGURE 12: THE NUMBER OF HOURS PER WEEK INVOLVED IN MODERATE ACTIVITY

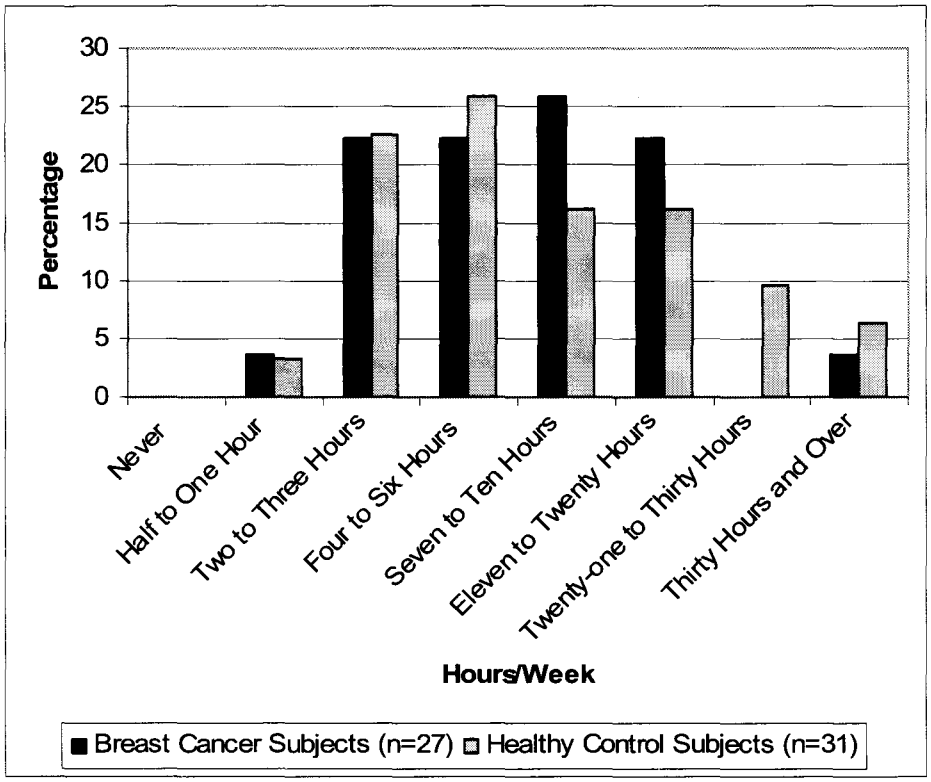
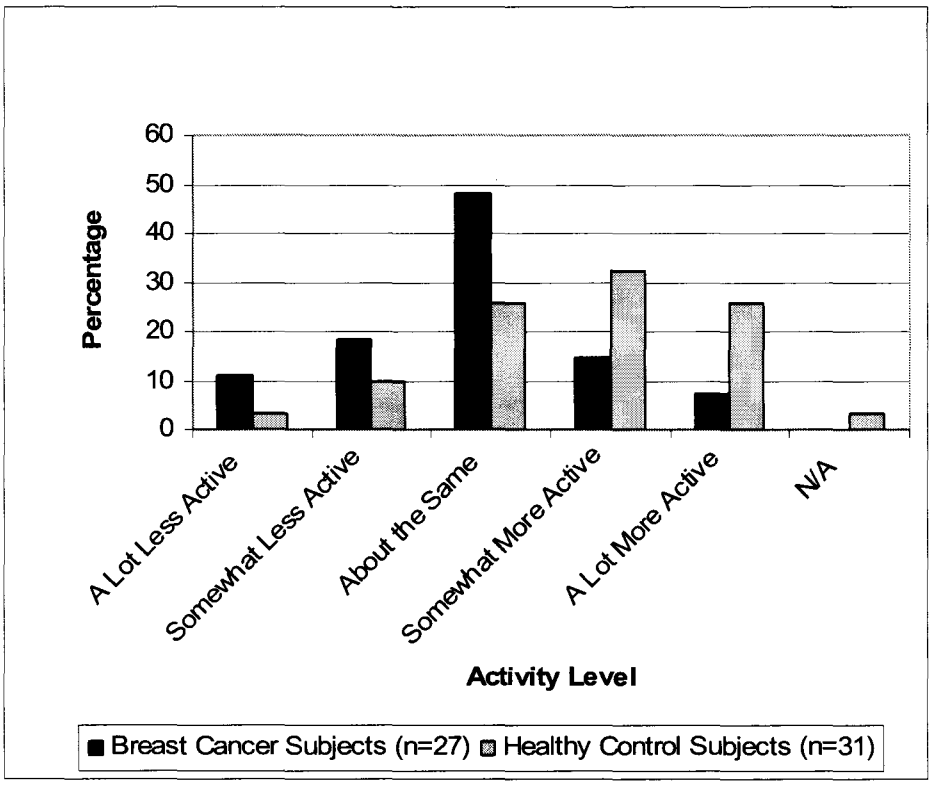


FIGURE 13: SUBJECTS PERCEIVED ACTIVITY LEVEL AS COMPARED WITH THEIR PEERS



4.2.2 Osteoporosis Knowledge

The mean score (SD) on the FOOQ was 14 (3) out of a possible 20 and the scores ranged from 6 to 19. The median score was 15, indicating that only half of the women responded correctly to at least 75% of the questions. When the study sample was divided into two subgroups, there was a statistically significant difference ($p<0.01$) between the mean scores for healthy postmenopausal women (15) and breast cancer subjects (13). There were a number of questions where more than 20% of all participants were either incorrect in their response to the question or did not know the answer. These questions included: whether low weight women have osteoporosis more than heavy women (41% scored incorrect; and 21% responded *don't know*); if the most important time to build bone strength is between 9 and 17 years of age (22% scored incorrect; and 17% responded *don't know*); if early menopause is a risk factor for osteoporosis (12% scored incorrect; and 33% responded *don't know*); and if replacing hormones after menopause can slow down bone loss (19% scored incorrect; and 40% responded *don't know*). The question which measured the highest *don't know* response was whether alcoholism is associated with the occurrence of osteoporosis (47% responded *don't know*). Interestingly, 99% of the participants either scored incorrectly or did not know if walking has a great effect on bone health.

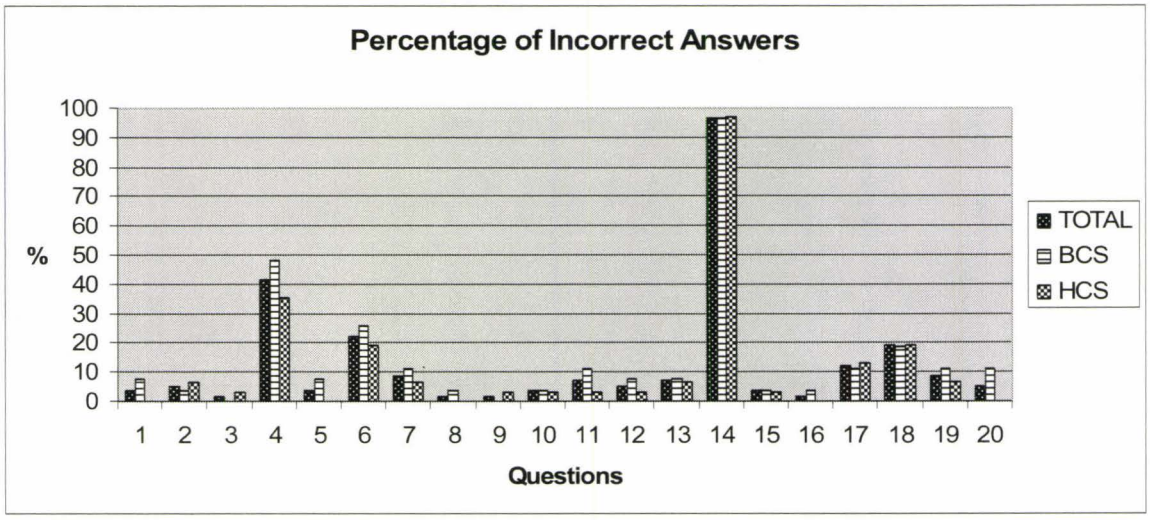
Overall, respondents had the lowest number of correct responses to items related to risk factors and preventative measures regarding the pathogenesis of osteoporosis. The percentage of incorrect and *don't know* responses to each question on the FOOQ is recorded in Table 14. Notably, breast cancer subjects responded *don't know* more often on all questions, with the exception of the question concerning whether after menopause women not

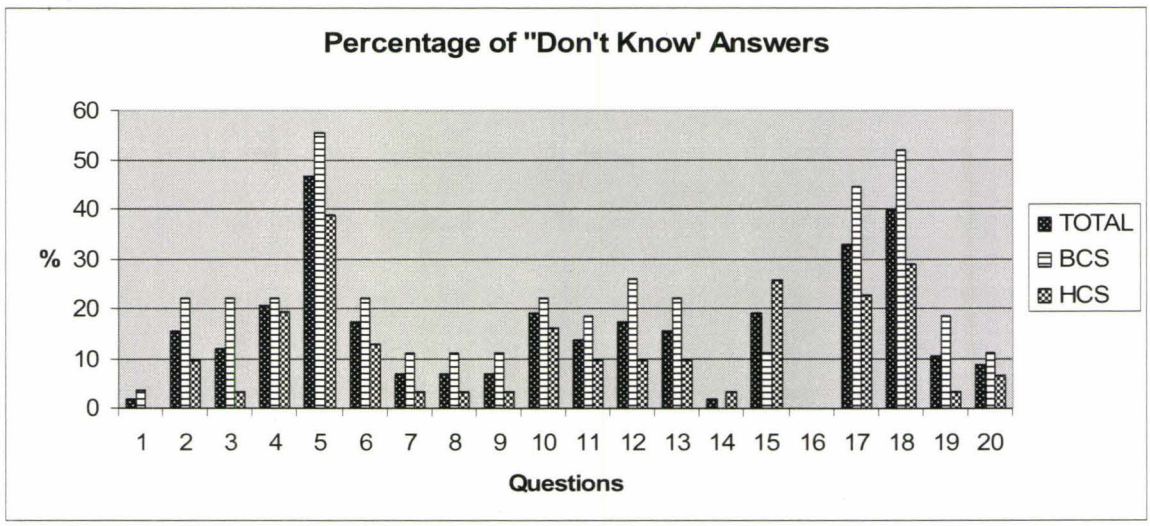
taking estrogen need about 1500 mg of calcium daily. A comparison of the percentage of questions answered either incorrectly or with a *don't know* response for breast cancer subjects (BCS) versus healthy control subjects (HCS) is shown in Figure 14.

TABLE 14: RESULTS FROM THE FACTS ON OSTEOPOROSIS QUIZ (FOOQ)

Item	Responses (%) (n = 58) Incorrect	Don't Know
1. *Physical activity increases the risk of osteoporosis.*	3	2
2. *High impact exercise (weight training) improves bone health.*	5	16
3. *Most people gain bone mass after 30 years of age.*	2	12
4. *Low weight women have osteoporosis more than heavy women.*	41	21
5. *Alcoholism is not linked to the occurrence of osteoporosis.*	3	47
6. *The most important time to build bone strength is between 9 and 17 years of age.*	22	17
7. *Normally, bone loss speeds up after menopause.*	9	7
8. *High caffeine combined with low calcium intake increases the risk of osteoporosis.*	2	7
9. *There are many ways to prevent osteoporosis.*	2	7
10. *Without preventative measures, 20% of women older than 50 years will have a fracture due to osteoporosis in their lifetime.*	3	19
11. *There are treatments for osteoporosis after it develops.*	7	14
12. *A lifetime of low intake of calcium and vitamin D does not increase the risk of osteoporosis.*	5	17
13. *Smoking does not increase the risk of osteoporosis.*	7	16
14. *Walking has a great effect on bone health.*	97	2
15. *After menopause, women not on estrogen need about 1500 mg of calcium (for example, 5 glasses of milk) daily.*	3	18
16. *Osteoporosis affects men and women.*	2	0
17. *Early menopause is not a risk factor for osteoporosis.*	12	33
18. *Replacing hormones after menopause cannot slow down bone loss.*	19	40
19. *Children 9 to 17 years of age get enough calcium from one glass of milk each day to prevent osteoporosis.*	9	10
20. *Family history of osteoporosis is not a risk factor for osteoporosis.*	5	9

FIGURE 14: SUB-GROUP RESULTS FOR THE FOOQ





4.3 pQCT Analysis of Trabecular and Cortical Bone at the Radius and Tibia in Healthy Postmenopausal Women and Women with a History of Breast Carcinoma

This sub-study was designed to detect and analyze any differences in bone density, mass, and geometry between healthy postmenopausal women and women recently diagnosed with breast carcinoma. The anthropometric characteristics of the subjects are given in Table 12. The sample population was described previously, in section *4.2.1.* In brief, there were 58 women recruited for this study, 31 healthy postmenopausal women without a history of breast carcinoma and 27 women recently diagnosed with stage I or stage II breast carcinoma. There were no significant differences among the breast cancer subjects for any of the measured variables with respect to their chemotherapy regimen. The mean (SD) age of all participants was 60 (8) years (range 46 to 81 years) and in the subgroups, 58 (7) for healthy postmenopausal women and 62 (9) for breast cancer subjects.

4.3.1 Distal Radius

The distance from the olecranon process to the ulnar styloid process was measured to the nearest 0.1 cm on the non-dominant forearm of each subject. Table 15 lists the pQCT variables measured at the 4% distal radius in the healthy postmenopausal subjects and the breast cancer subjects. Also given in the table, where applicable, is the statistical significance of the difference between the measured parameters in these two subgroups. One scan at the 4% site of a breast cancer subject displayed an inadequately high level of motion artifact; this scan had to be excluded from further analysis for this reason. Therefore, at the 4% radius site, the sample size of breast cancer subjects was n=26.

TABLE 15: BONE VARIABLES AT THE 4% RADIUS

Variable[a]	All Subjects (n = 57)	Healthy Post-Menopausal Subjects (n = 31)	Breast Cancer Subjects (n = 26)
Density (mg/cm^3)			
TOT_DEN	341 (9.5)	359 (12.7)	319 (13.4)*
TRAB_DEN	175 (5.0)	182 (6.9)	167 (6.9)
Mass (mg)			
TOT_CNT	97 (2.1)	103 (2.9)	91 (2.6)**
TRAB_CNT	23 (0.8)	24 (1.2)	22 (0.9)
Area (mm^2)			
TOT_A	292 (6.3)	292 (8.1)	292 (10.0)

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; TRAB_DEN, trabecular density; TOT_CNT, total content; TRAB_CNT, trabecular content; TOT_A, total area; *$p<0.05$ and **$p<0.01$ for 2 sample t-test

The mean value for TOT_DEN at the distal radius was 359 mg/cm^3 in the healthy control subjects; results were significantly lower ($p<0.05$) in the breast cancer subjects at 319 mg/cm^3. The results for TOT_CNT were also significantly different ($p<0.01$) between the two groups with mean values of 103 mg and 91 mg respectively for the healthy and breast cancer subjects. The values for TRAB_DEN, TRAB_CNT and TOT_A were also lower in the breast cancer subjects, but not to a statistically significant degree. Figures 15 through 19 display the normal probability plots for all the pQCT variables measured at the distal radius with separate data distributions displayed for each cohort of subjects. These figures also display the 95% confidence intervals for each data set and the Anderson Darling goodness of fit data for each variable. There were no significant outliers detected for either subject group at the distal radius. For the variables that were significantly different between the two sample populations, specifically TOT_DEN and TOT_CNT, regression analysis was conducted on the data to determine any relationship between the pQCT measurement parameters and the

anthropometric characteristics of the study sample. BMI accounted for less than 10% of the variability of the data sets; R^2=9.6% for TOT_DEN and R^2=9.9% for TOT_CNT. The data also indicate that less than 5% of the variation in TOT_DEN (R^2=3.4%) and TOT_CNT (R^2=4.6%) is explained by the variation in age; and an even smaller percentage can be explained by the variation in age at menopause (AAM)(R^2=0% for both variables), years since menopause (YSM)(R^2=1.6% TOT_DEN, R^2=2.7% TOT_CNT), or number of children (NOC)(R^2=0.2% TOT_DEN, R^2=4.4% TOT_CNT). The largest variation in the TOT_CNT data set could be accounted for by the time on Anastrozole (TOA) (R^2=5.3% TOT_DEN, R^2=12.9% TOT_CNT). The regression analysis for TOT_DEN and TOT_CNT, including the regression equations and R^2 values for the variables at the 4% radius site are displayed in Figures 20 through 31.

FIGURE 15: NORMAL PROBABILITY PLOT OF TOT_DEN AT THE 4% RADIUS

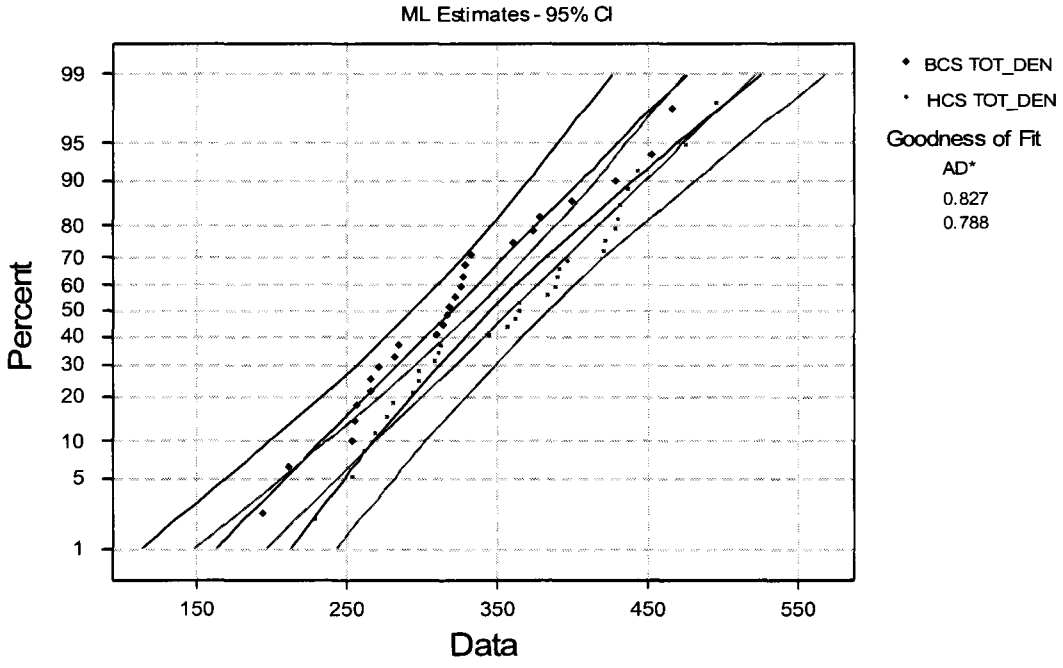


FIGURE 16: NORMAL PROBABILITY PLOT OF TRAB_DEN AT THE 4% RADIUS

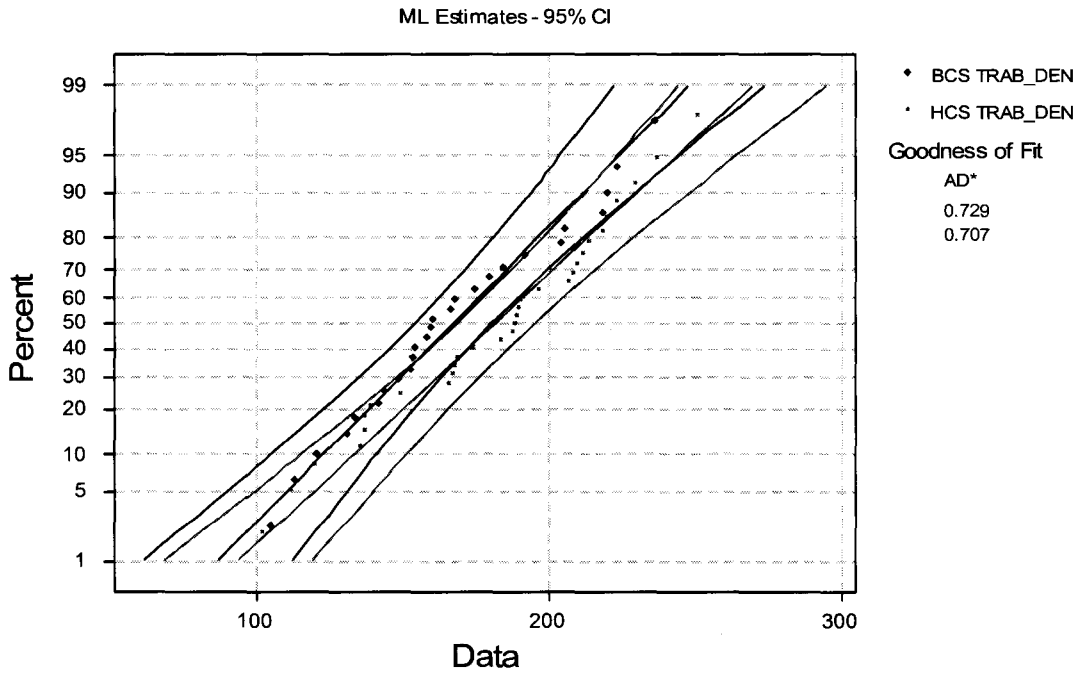


FIGURE 17: NORMAL PROBABILITY PLOT OF TOT_CNT AT THE 4% RADIUS

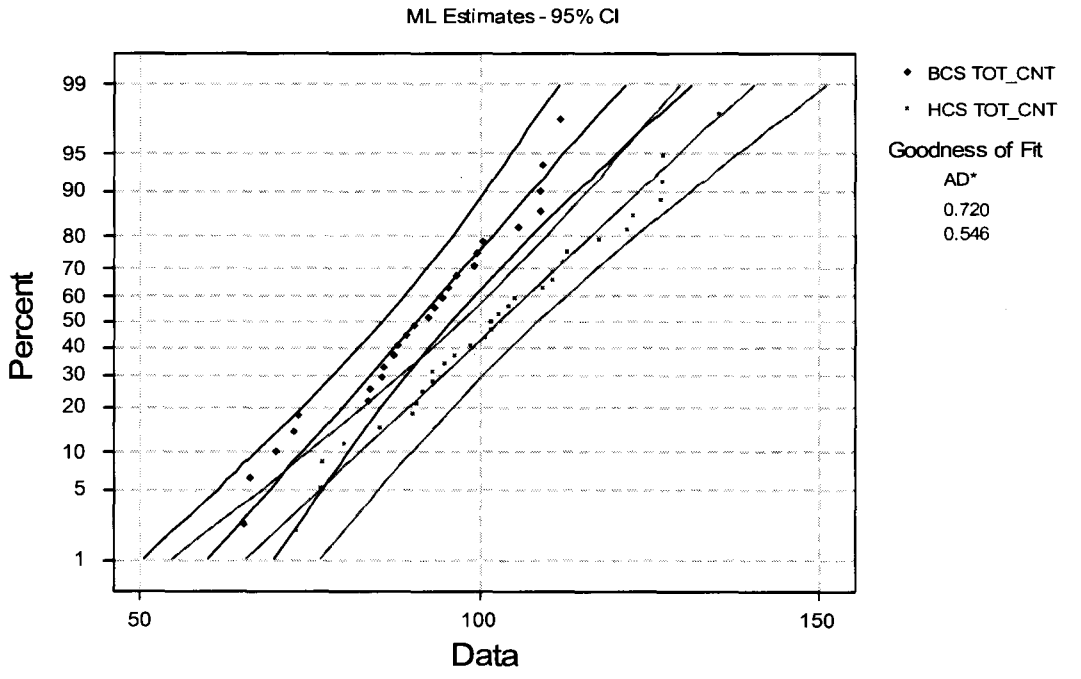


FIGURE 18: NORMAL PROBABILITY PLOT OF TRAB_CNT AT THE 4% RADIUS

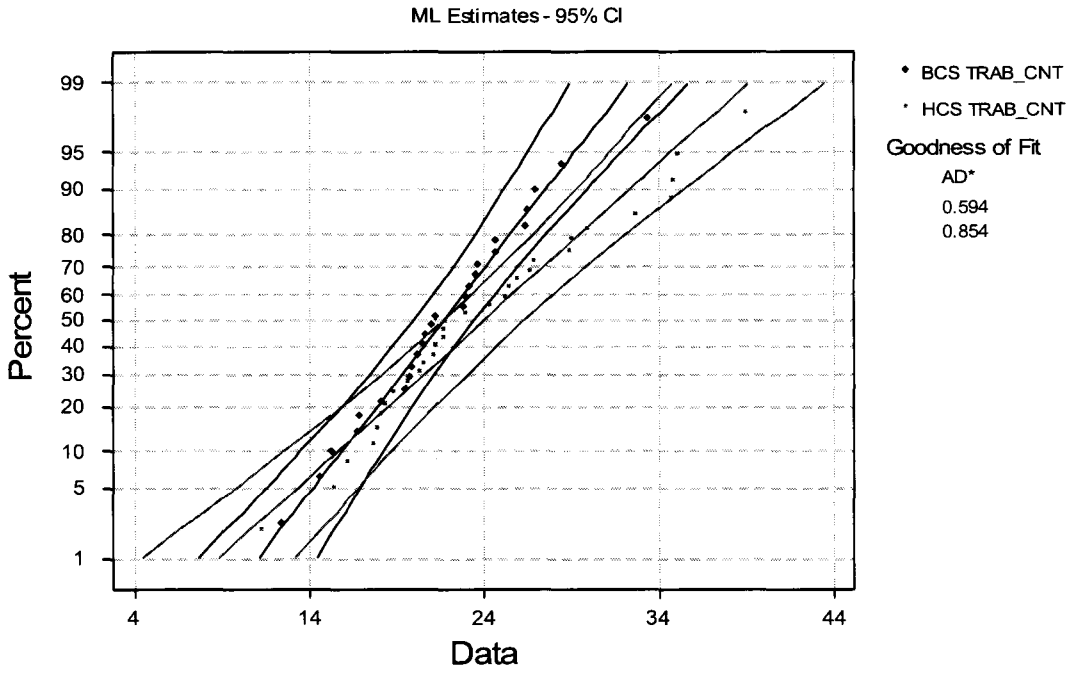


FIGURE 19: NORMAL PROBABILITY PLOT OF TOT_A AT THE 4% RADIUS

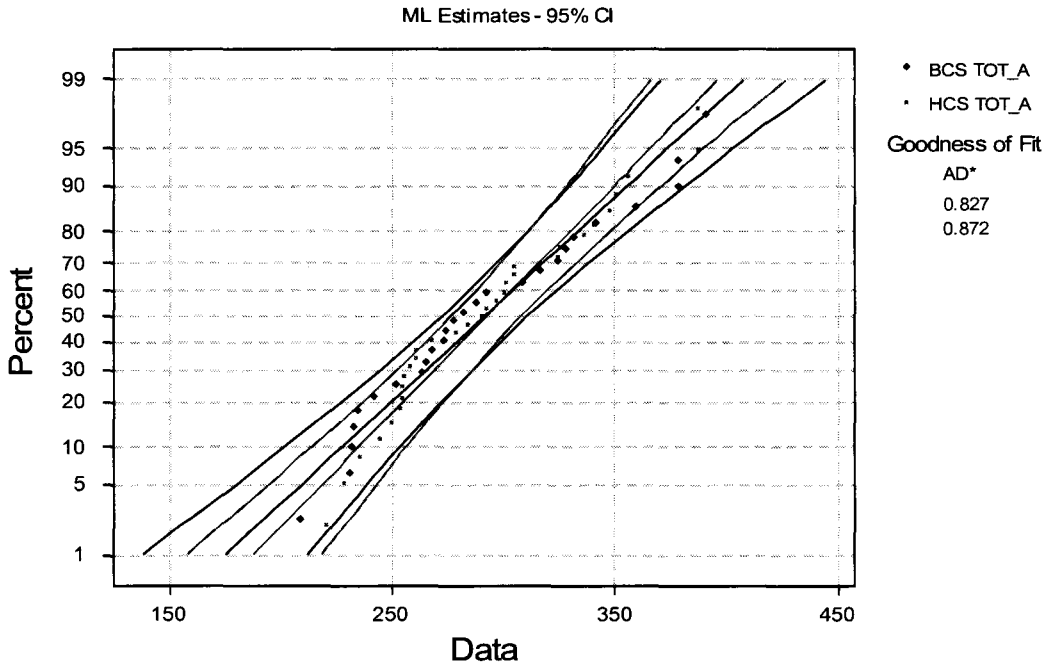


FIGURE 20: REGRESSION ANALYSIS OF TOT_DEN AND BMI AT THE 4% RADIUS

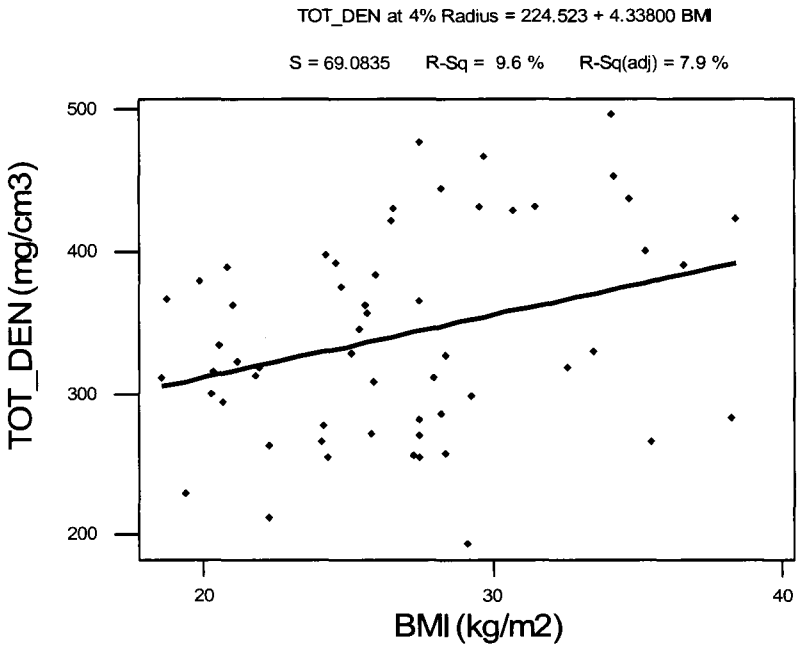


FIGURE 21: REGRESSION ANALYSIS OF TOT_CNT AND BMI AT THE 4% RADIUS

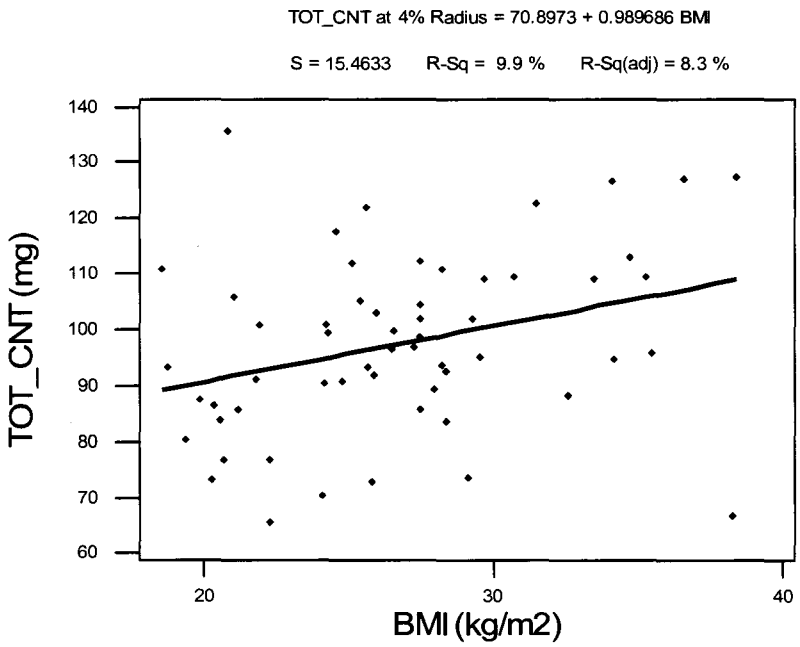


FIGURE 22: REGRESSION ANALYSIS OF TOT_DEN AND AGE AT THE 4% RADIUS

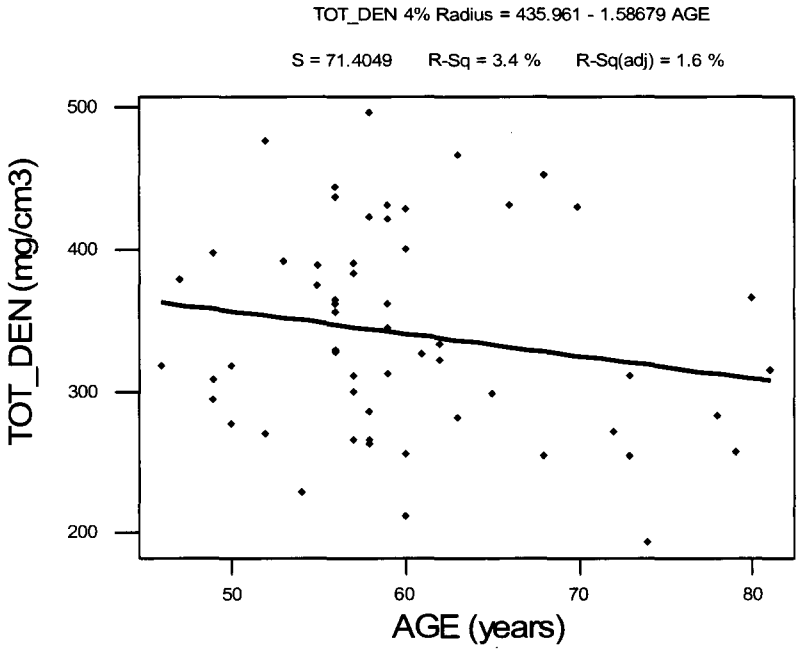


FIGURE 23: REGRESSION ANALYSIS OF TOT_CNT AND AGE AT THE 4% RADIUS

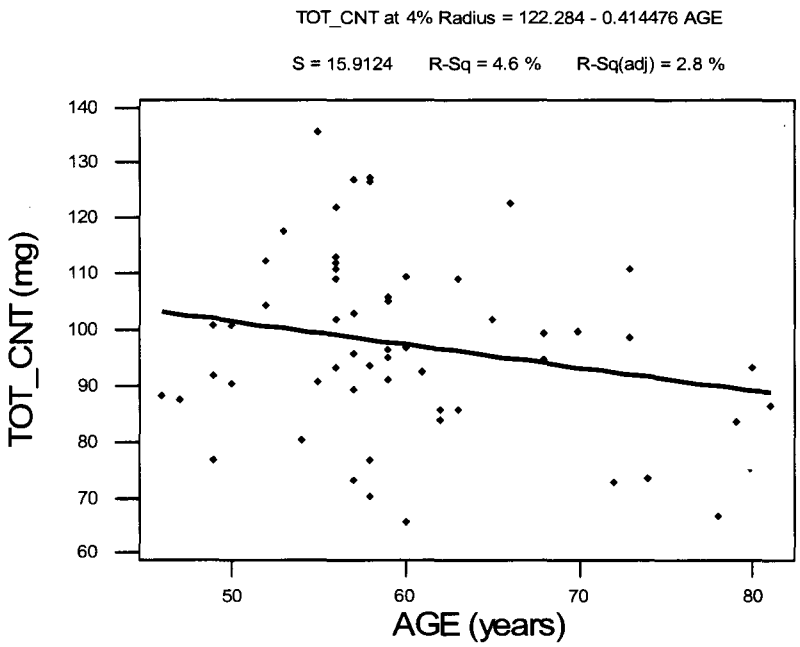


FIGURE 24: REGRESSION ANALYSIS OF TOT_DEN AND AAM AT THE 4% RADIUS

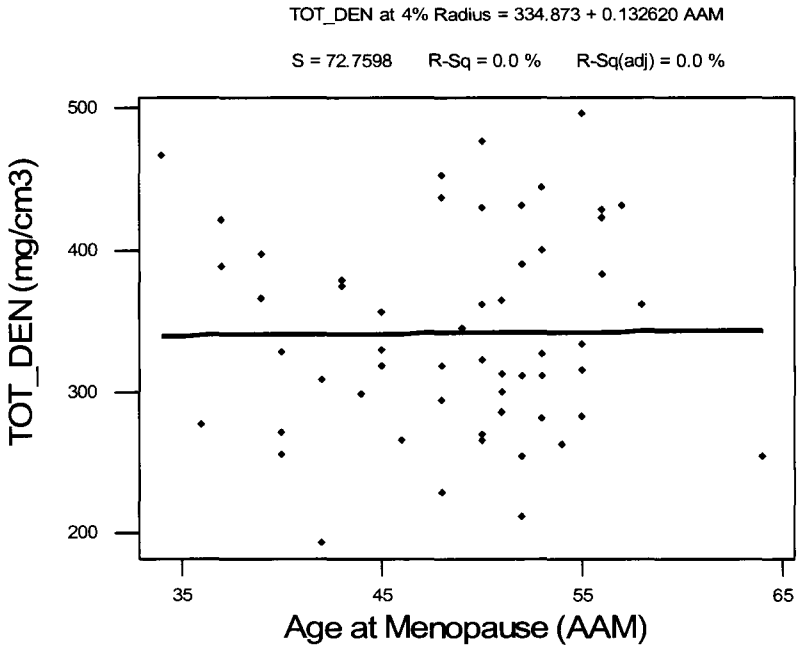


FIGURE 25: REGRESSION ANALYSIS OF TOT_CNT AND AAM AT THE 4% RADIUS

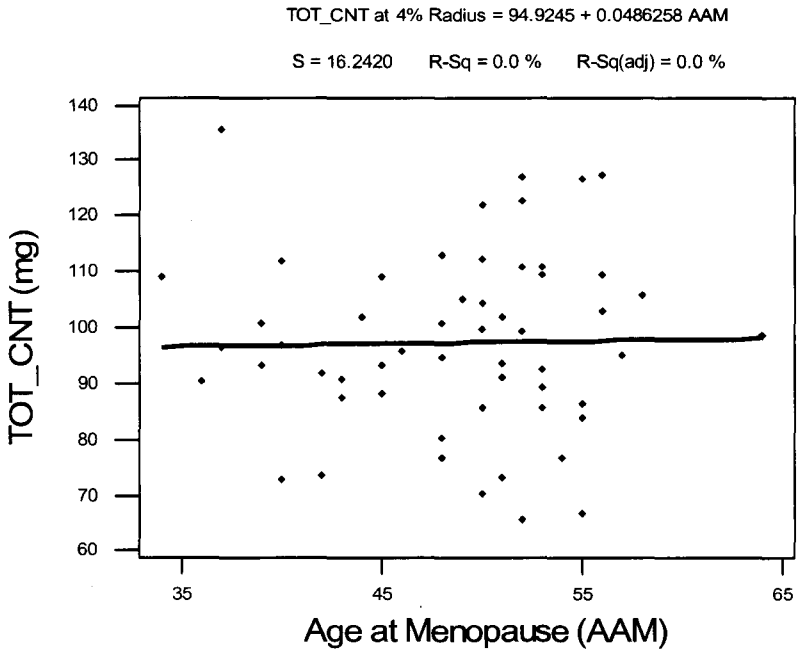


FIGURE 26: REGRESSION ANALYSIS OF TOT_DEN AND YSM AT THE 4% RADIUS

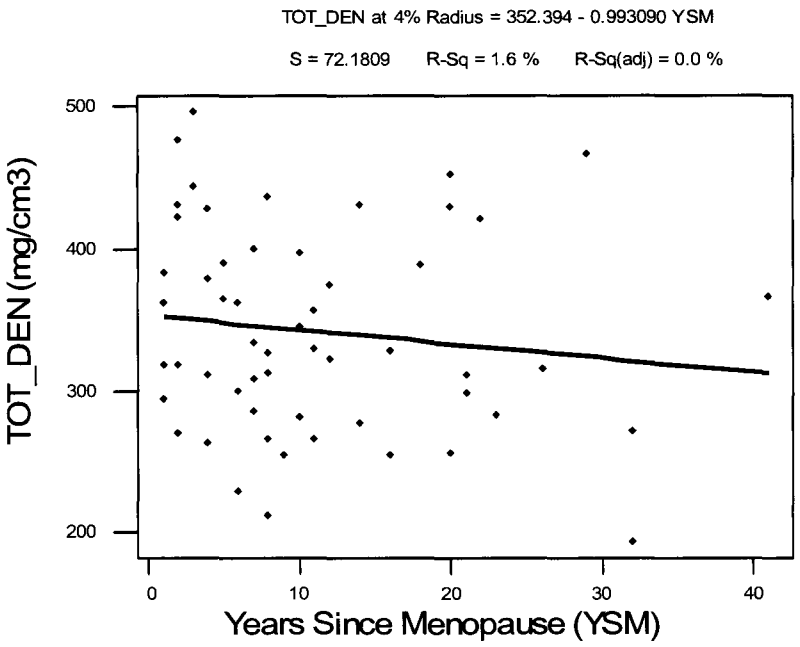


FIGURE 27: REGRESSION ANALYSIS OF TOT_CNT AND YSM AT THE 4% RADIUS

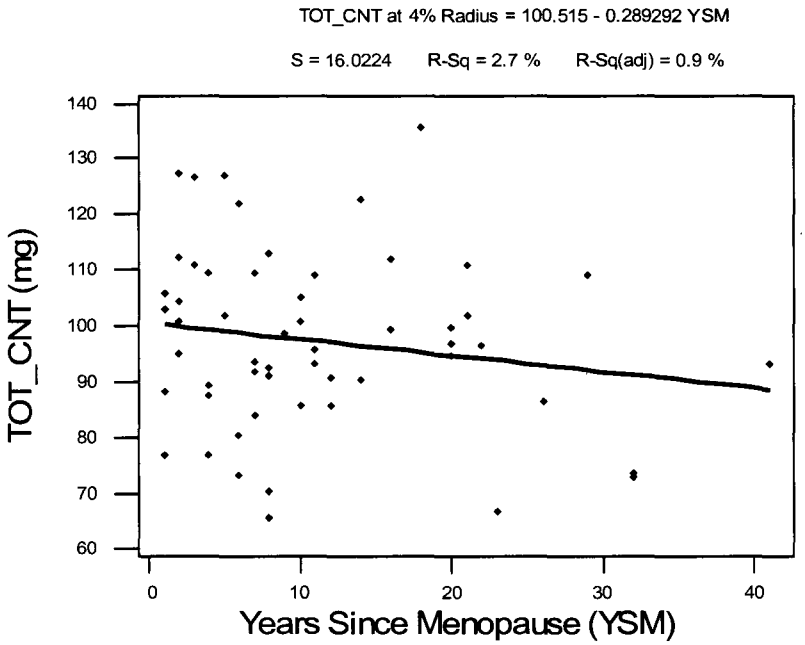


FIGURE 28: REGRESSION ANALYSIS OF TOT_DEN AND NOC AT THE 4% RADIUS

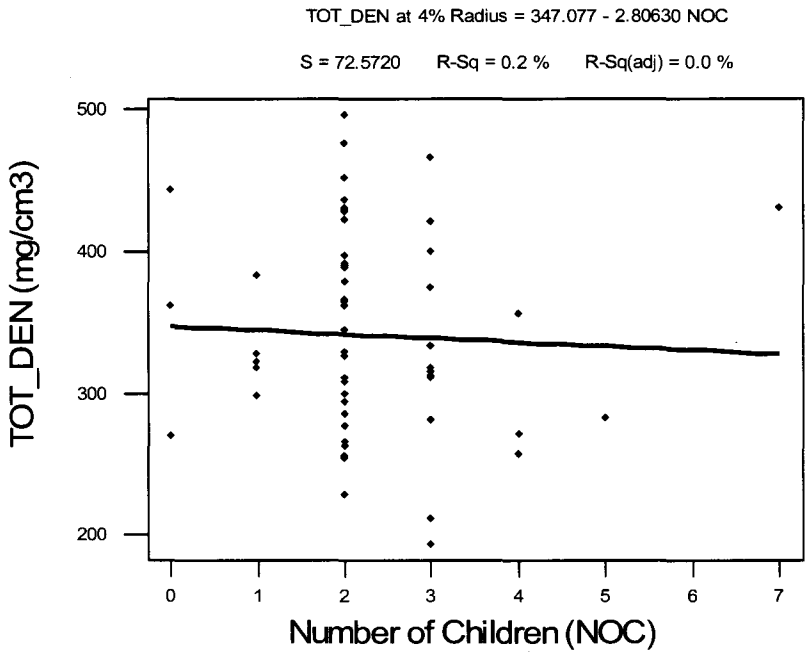


FIGURE 29: REGRESSION ANALYSIS OF TOT_CNT AND NOC AT THE 4% RADIUS

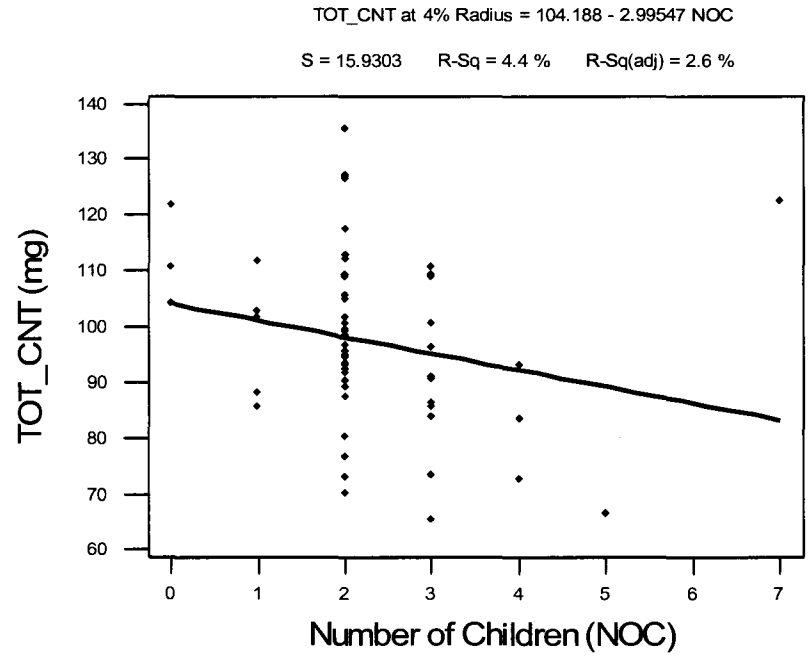


FIGURE 30: REGRESSION ANALYSIS OF TOT_DEN AND TOA AT THE 4% RADIUS

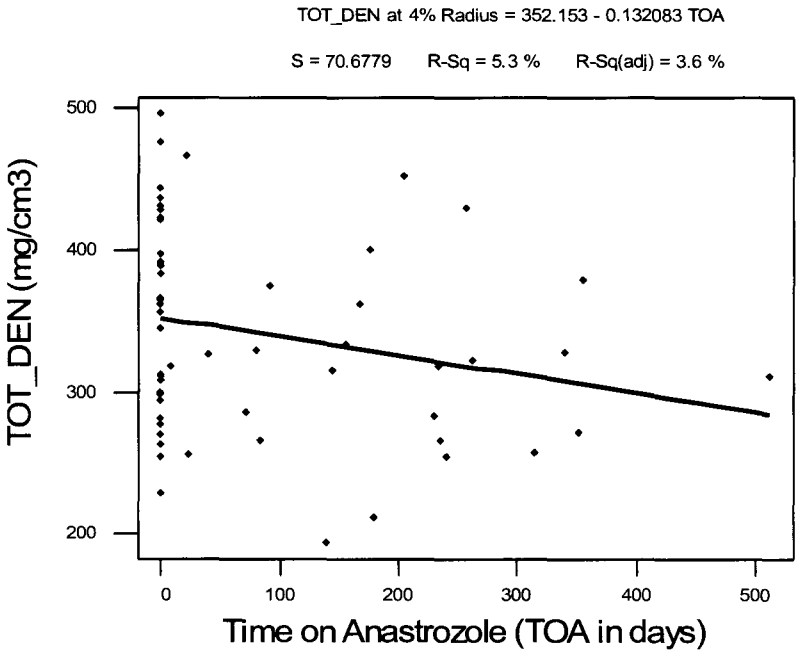
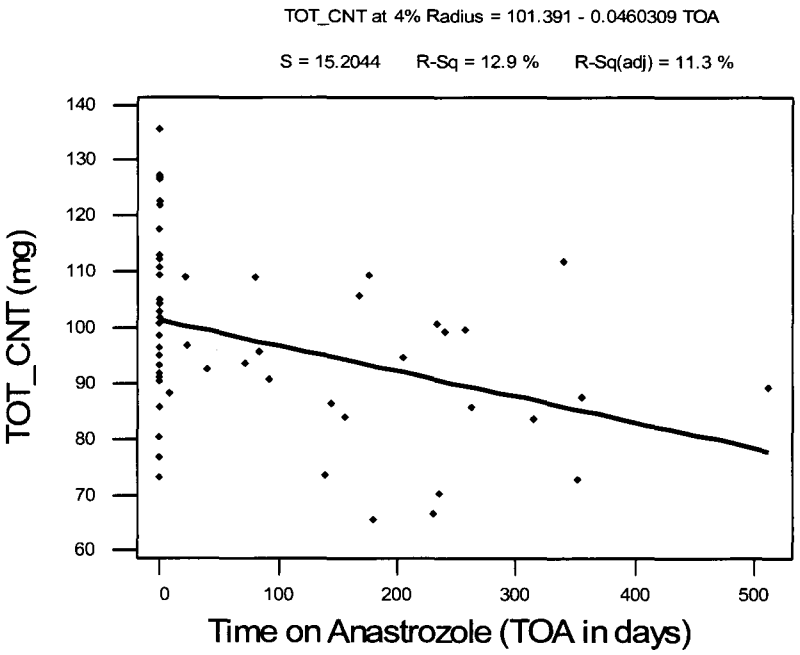


FIGURE 31: REGRESSION ANALYSIS OF TOT_CNT AND TOA AT THE 4% RADIUS



4.3.2 Distal Tibia

The length of the bone from the distal end of the medial malleolus to the medial knee joint cleft was measured. Scans were performed on the non-dominant tibia of each subject using the standard imaging procedure as described previously in section *3.3*. Table 16 displays the pQCT variables measured at the 4% distal tibia in healthy postmenopausal subjects (n=31) and breast cancer subjects (n=27).

TABLE 16: BONE VARIABLES AT THE 4% TIBIA

Variable[a]	All Subjects (n = 58)	Healthy Post-Menopausal Subjects (n = 31)	Breast Cancer Subjects (n = 27)
Density (mg/cm^3)			
TOT_DEN	287 (5.2)	297 (7.5)	276 (6.6)*
TRAB_DEN	226 (4.7)	230 (6.9)	222 (6.4)
Mass (mg)			
TOT_CNT	286 (5.0)	291 (7.2)	281 (6.8)
TRAB_CNT	102 (2.3)	102 (3.3)	102 (3.2)
Area (mm^2)			
TOT_A	1003 (14)	986 (19)	1022 (21)

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; TRAB_DEN, trabecular density; TOT_CNT, total content; TRAB_CNT, trabecular content; TOT_A, total area; *p< 0.05 for 2 sample t-test

The mean value for TOT_DEN at the distal tibia was 297 mg/cm^3 in the healthy control subjects; results were significantly lower ($p<0.05$) in the breast cancer subjects at 276 mg/cm^3. The results for TRAB_DEN and TOT_CNT were also slightly lower in the breast cancer population. Interestingly, the values for TRAB_CNT were the same for both populations at 102 mg, and TOT_A values were higher for the breast cancer subjects at 1022 mm^2 versus 986 mm^2 for the control subjects. Figures 32 through 36 display the normal probability plots and 95% confidence intervals for all the variables measured at the distal tibia

as well as the data distribution for both cohorts of subjects.

Regression analysis was conducted to determine any relationship between the only statistically significantly lower variable in the breast cancer subjects, TOT_DEN, and the anthropometric characteristics of the study sample. Approximately 16% of the variation in TOT_DEN (R^2=16.4%) can be explained by BMI. Subjects age (R^2=0.1%), AAM (R^2=0.9%), YSM (R^2=0.5%), NOC (R^2=3.3%), accounted for a very small percentage of variability in TOT_DEN at the distal tibia. TOA (R^2=4.3%), was responsible for the largest negative variability in the data set at this site. The regression analysis for TOT_DEN, including the regression equation and R^2 values for the variables at the 4% tibia site are shown in Figures 37 through 42.

FIGURE 32: NORMAL PROBABILITY PLOT OF TOT_DEN AT THE 4% TIBIA

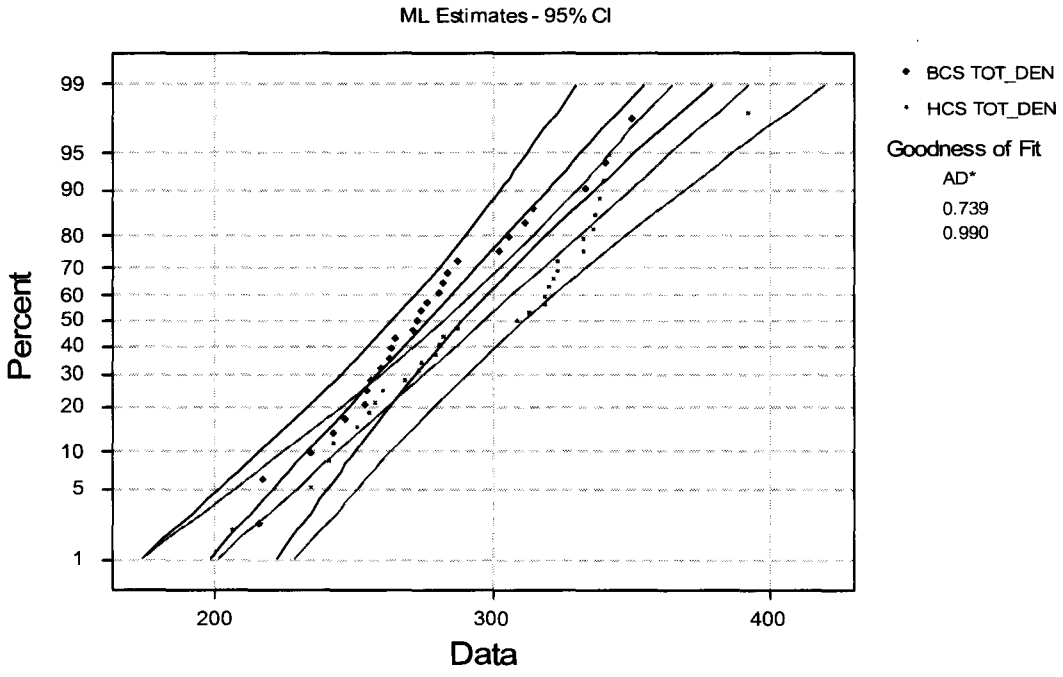


FIGURE 33: NORMAL PROBABILITY PLOT OF TRAB_DEN AT THE 4% TIBIA

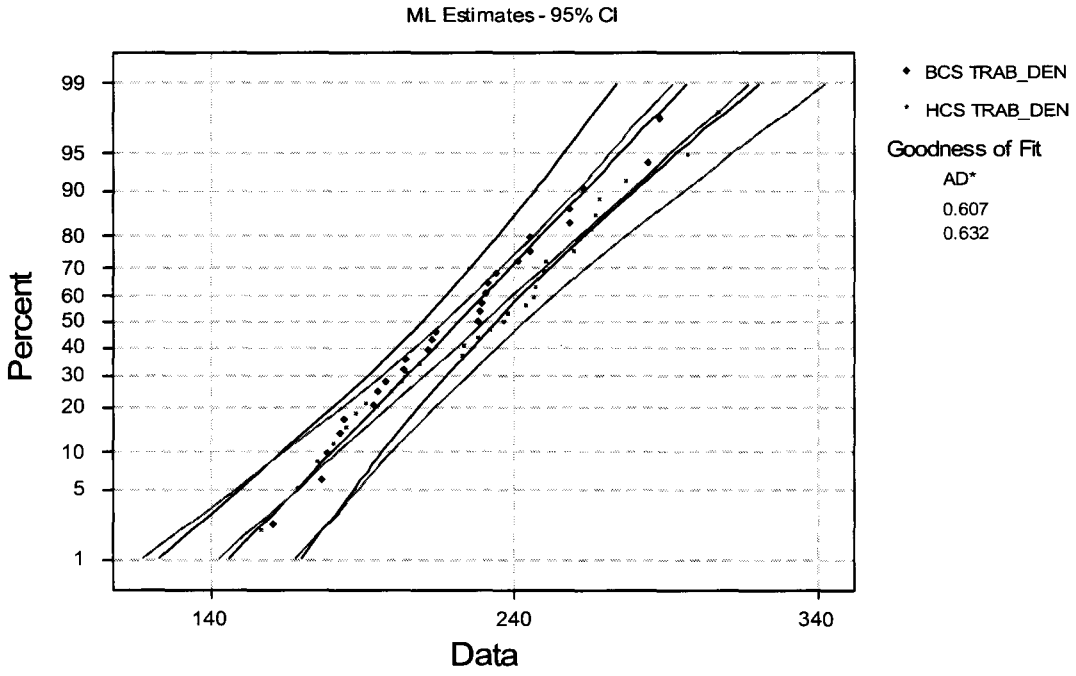


FIGURE 34: NORMAL PROBABILITY PLOT OF TOT_CNT AT THE 4% TIBIA

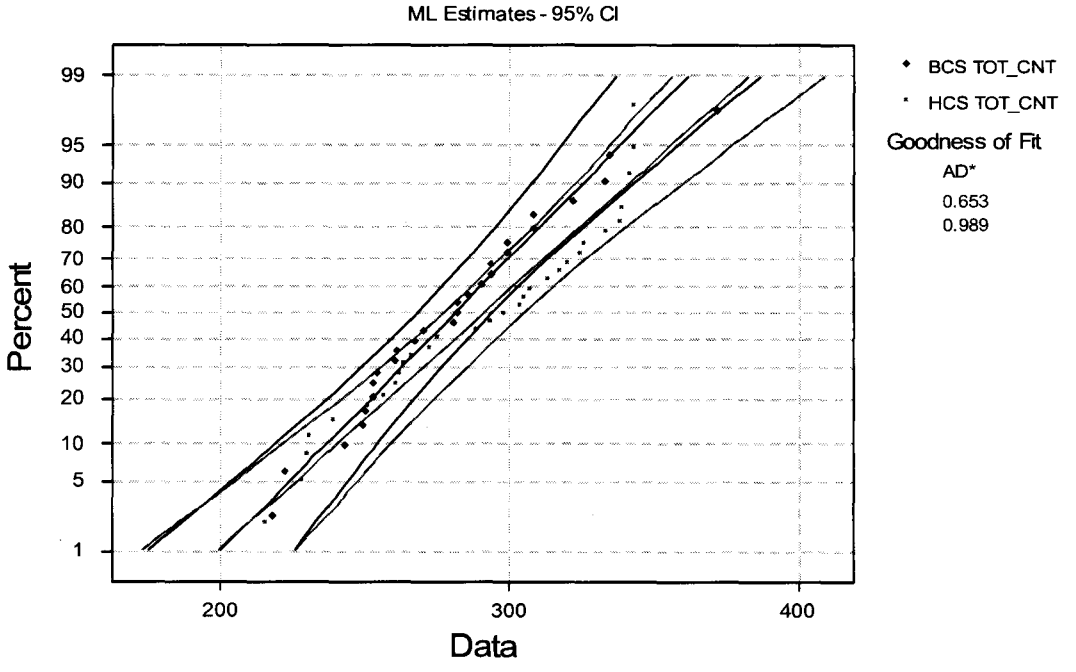


FIGURE 35: NORMAL PROBABILITY PLOT OF TRAB_CNT AT THE 4% TIBIA

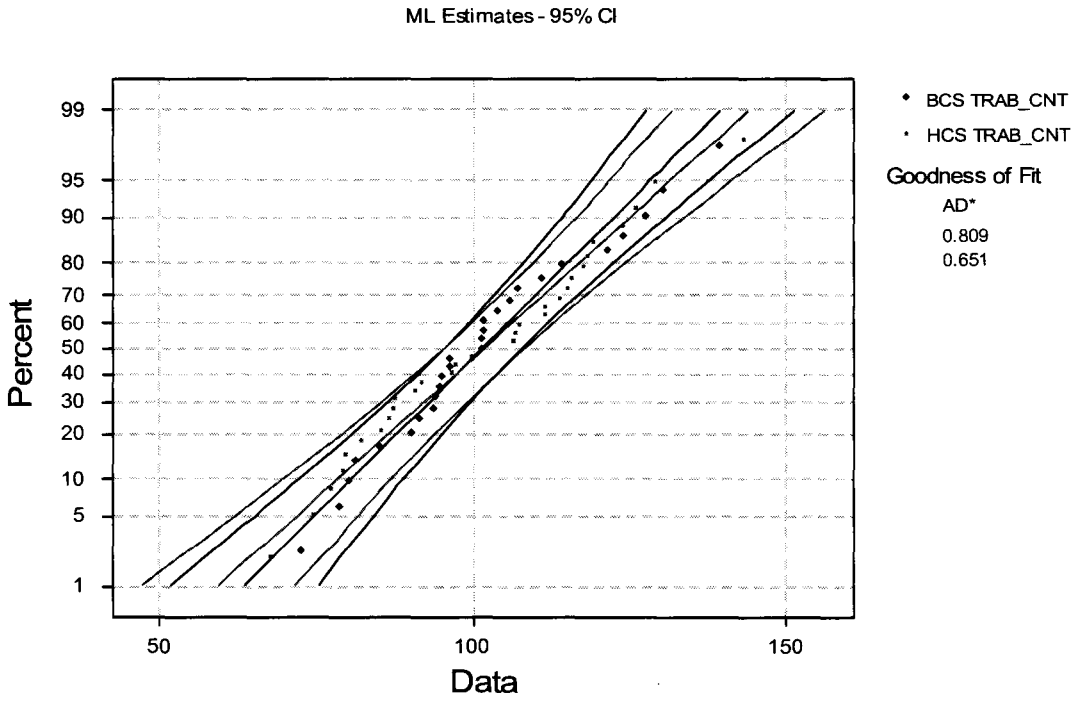


FIGURE 36: NORMAL PROBABILITY PLOT OF TOT_A AT THE 4% TIBIA

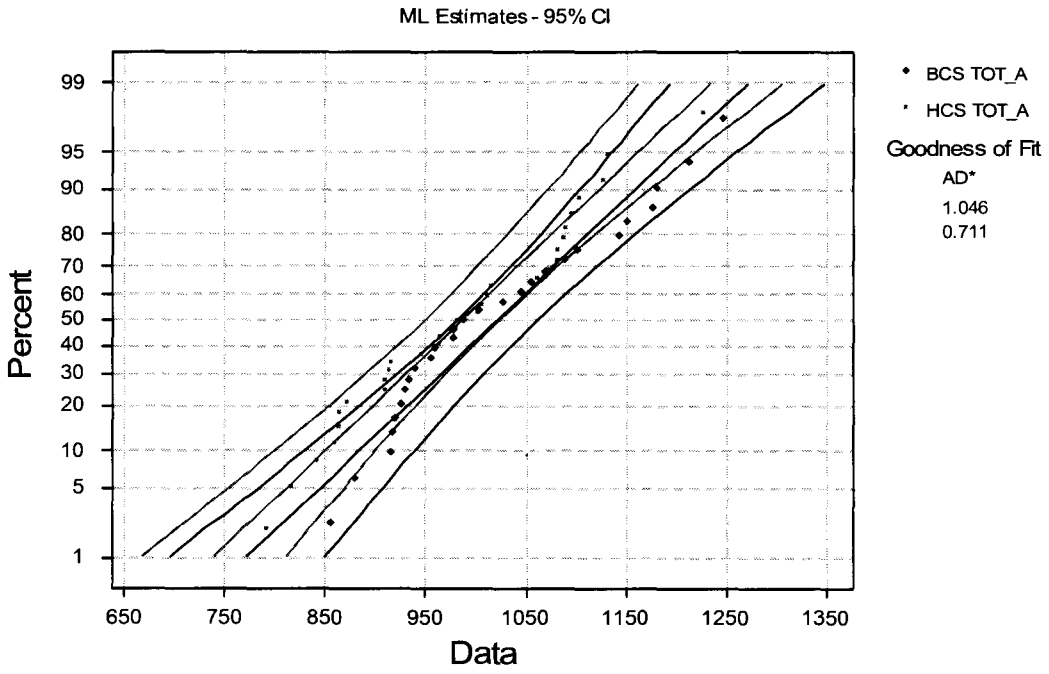


FIGURE 37: REGRESSION ANALYSIS OF TOT_DEN AND BMI AT THE 4% TIBIA

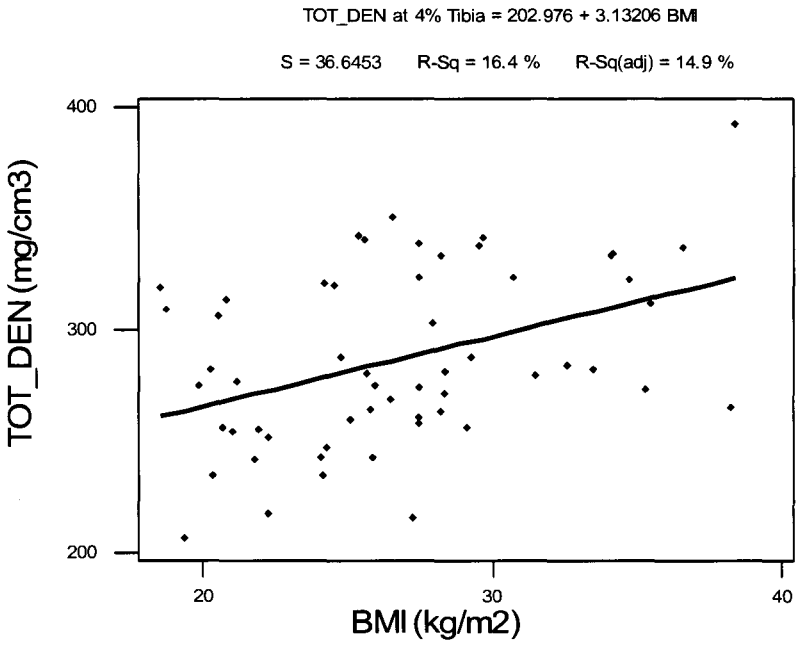


FIGURE 38: REGRESSION ANALYSIS OF TOT_DEN AND AGE AT THE 4% TIBIA

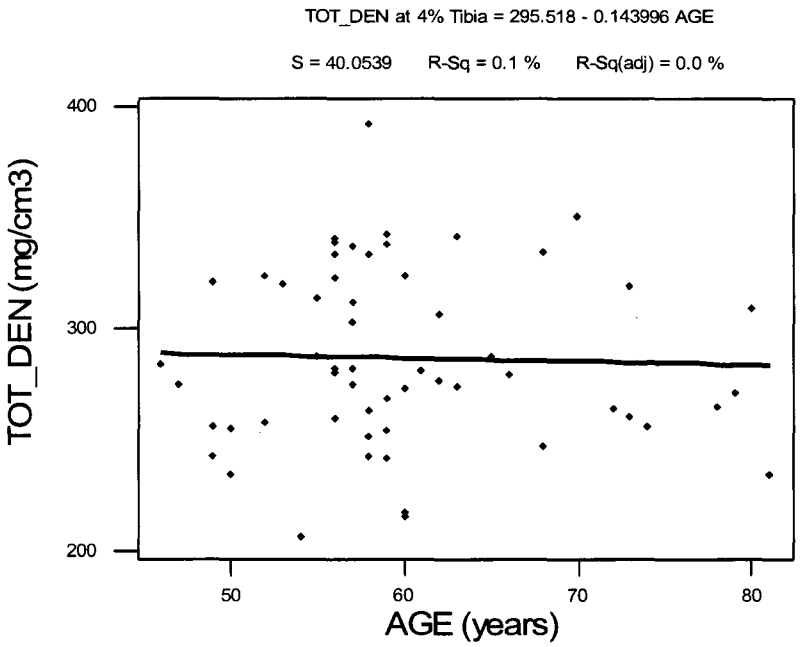


FIGURE 39: REGRESSION ANALYSIS OF TOT_DEN AND AAM AT THE 4% TIBIA

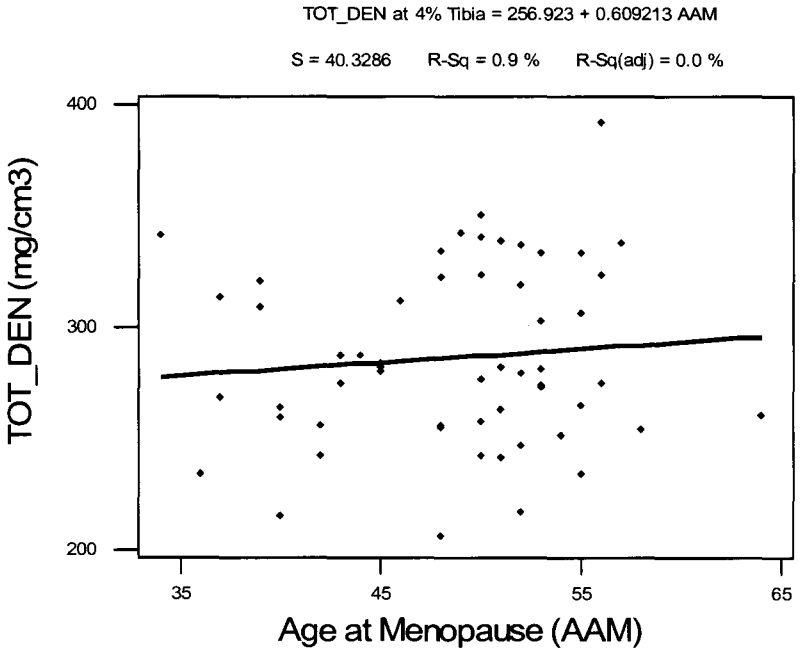


FIGURE 40: REGRESSION ANALYSIS OF TOT_DEN AND YSM AT THE 4% TIBIA

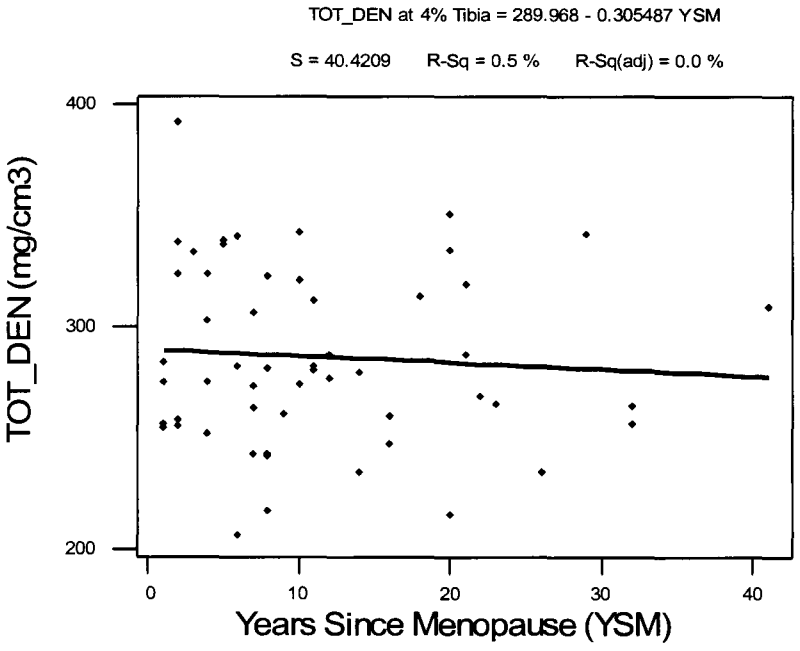


FIGURE 41: REGRESSION ANALYSIS OF TOT_DEN AND NOC AT THE 4% TIBIA

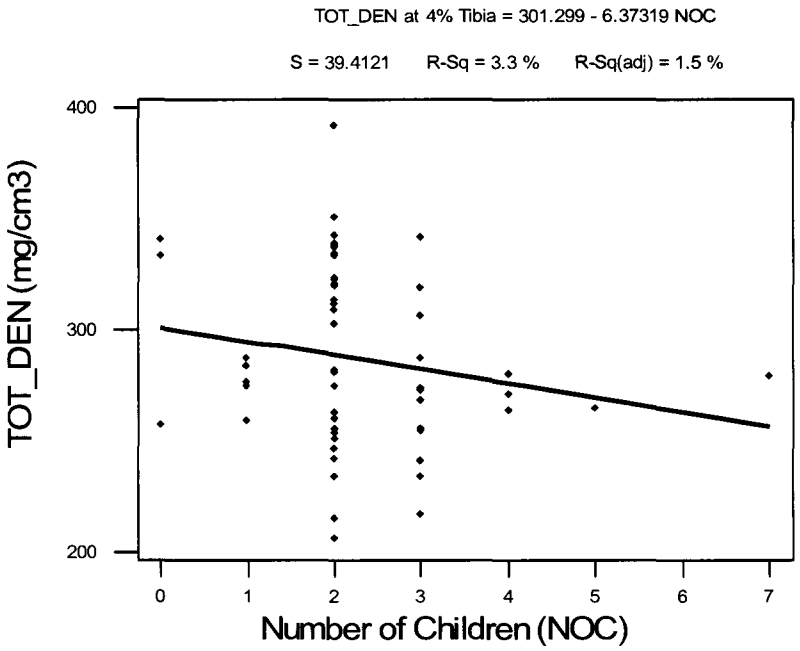
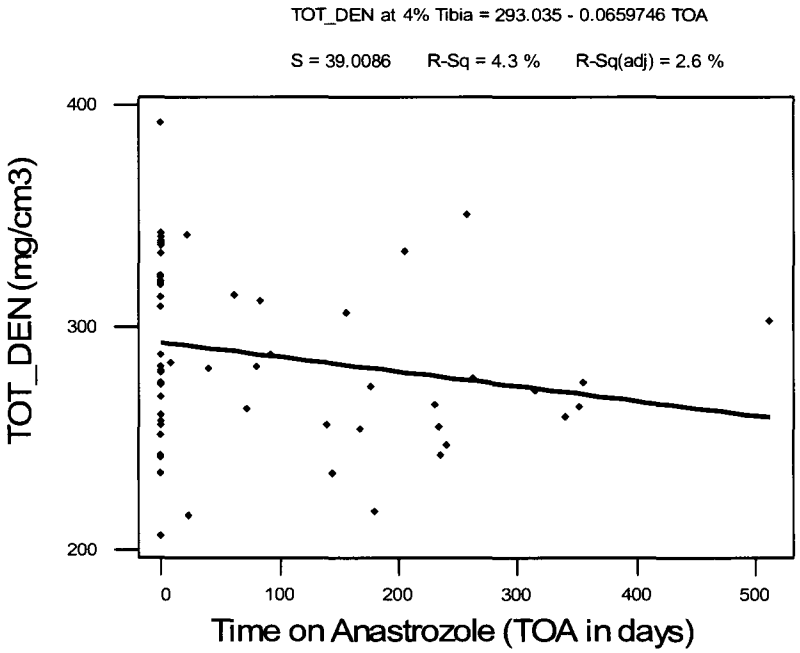


FIGURE 42: REGRESSION ANALYSIS OF TOT_DEN AND TOA AT THE 4% TIBIA



4.3.3 Diaphyseal Radius

Table 17 displays the descriptive statistics of pQCT derived density, mass, area, and geometry at the 20% diaphyseal site of the non-dominant radius. Scans were performed on the non-dominant radius of each subject using the standard imaging procedure as described previously in section *3.3*. At this site, all images were analyzed; with the study sample including n=31 healthy postmenopausal women and n=27 breast cancer subjects. Also given in the table, where applicable, is the statistical significance of the difference between the measured parameters in the healthy and breast cancer subjects.

TABLE 17: BONE AND GEOMETRIC VARIABLES AT THE 20% RADIUS

Variable[a]	All Subjects (n = 58)	Healthy Post-Menopausal Subjects (n = 31)	Breast Cancer Subjects (n = 27)
Density (mg/cm^3)			
TOT_DEN	867 (16.1)	895 (21.1)	834 (23.4)
CRT_DEN	1250 (5.5)	1270 (6.9)	1227 (6.5)**
Mass (mg)			
TOT_CNT	92 (1.5)	95 (1.9)	89 (2.2)*
CRT_CNT	86 (1.6)	89 (1.9)	82 (2.4)*
Area (mm^2)			
CRT_A	69 (1.1)	70 (1.3)	67 (1.8)
Geometry			
CRT_THK *(mm)*	2.4 (0.1)	2.4 (0.1)	2.3 (0.1)
PERI_CIR *(mm)*	37 (0.4)	37 (0.6)	37 (0.5)
ENDO_CIR *(mm)*	22 (0.6)	21 (0.8)	23 (0.9)
PMI *(mm^3)*	1534 (54)	1558 (74)	1507 (80)
PMR *(mm^3)*	203 (5.1)	206 (6.6)	200 (7.8)
SSI *(mm^3)*	211 (5.3)	217 (6.8)	204 (8.3)

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; CRT_DEN, cortical density; TOT_CNT, total content; CRT_CNT, cortical content; TOT_A, total area; CRT-A, cortical area; CRT-THK, cortical thickness; ENDO_CIR, endosteal circumference; PERI_CIR, periosteal circumference; PMI; polar moment of inertia; PMR, polar moment of resistance; SSI, stress-strain index; *p<0.05 and **p< 0.01 for 2 sample t-test

At this diaphyseal site, the TOT_CNT and CRT_CNT were significantly lower ($p<0.05$) in the breast cancer subjects. The mean TOT_CNT measured 95 mg in the control subjects and 89 in the breast cancer subjects. The mean values for CRT_CNT were 89 mg and 82 mg for each group respectively. The mean CRT_DEN values were also significantly lower ($p<0.01$) in the breast cancer subjects, at 1227 mg/cm^3 as compared with 1270 mg/cm^3 for the healthy control subjects. Conversely, TOT_DEN was not significantly different between the two groups. The area and geometry values were similar for both groups. Normality of the data sets was assessed and the normal distribution plots, the 95% confidence intervals for all the variables measured at the diaphyseal radius, as well as the separate data distributions for both cohorts of subjects are displayed for each pQCT variable in Figures 43 through 53. To further explore the relationship between the anthropometric characteristics of the study sample and the pQCT variables, regression analysis was conducted for CRT_DEN, TOT_CNT, and CRT_CNT. These data demonstrate that at the 20% radius, the time on Anastrozole (R^2=19.5% for CRT_DEN, R^2=11.2% for CRT_CNT and R^2=11.1% for TOT_CNT) and age of the study participants (R^2=16.1% for CRT_DEN, R^2=17.9% for CRT_CNT and R^2=13.7% for TOT_CNT) accounted for the highest percentage of variability among anthropometric factors in the data sets. Variation due to YSM was next at R^2=12.3% for CRT_DEN, R^2=12.7% for CRT_CNT, and R^2=9.7% for TOT_CNT. BMI and AAM did not account for any of the variability for each parameter. Notably, at the 20% radius, the subjects' NOC had the highest R^2 values of all the sites measured at R^2=4.8% for CRT_DEN, R^2=9.3% for CRT_CNT, and R^2=10% for TOT_CNT. The regression analysis, including the regression equation and R^2 values for the measured pQCT variables at the 20% diaphyseal radius site are shown in Figures 54 through 71.

FIGURE 43: NORMAL PROBABILITY PLOT OF TOT_DEN AT THE 20% RADIUS

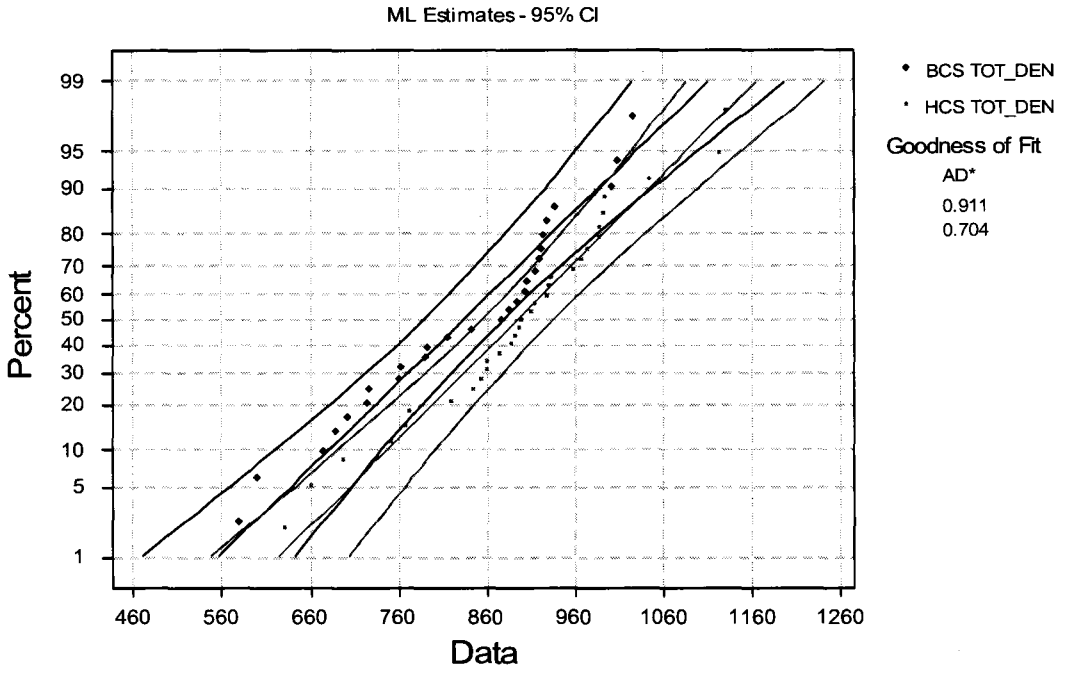


FIGURE 44: NORMAL PROBABILITY PLOT OF CRT_DEN AT THE 20% RADIUS

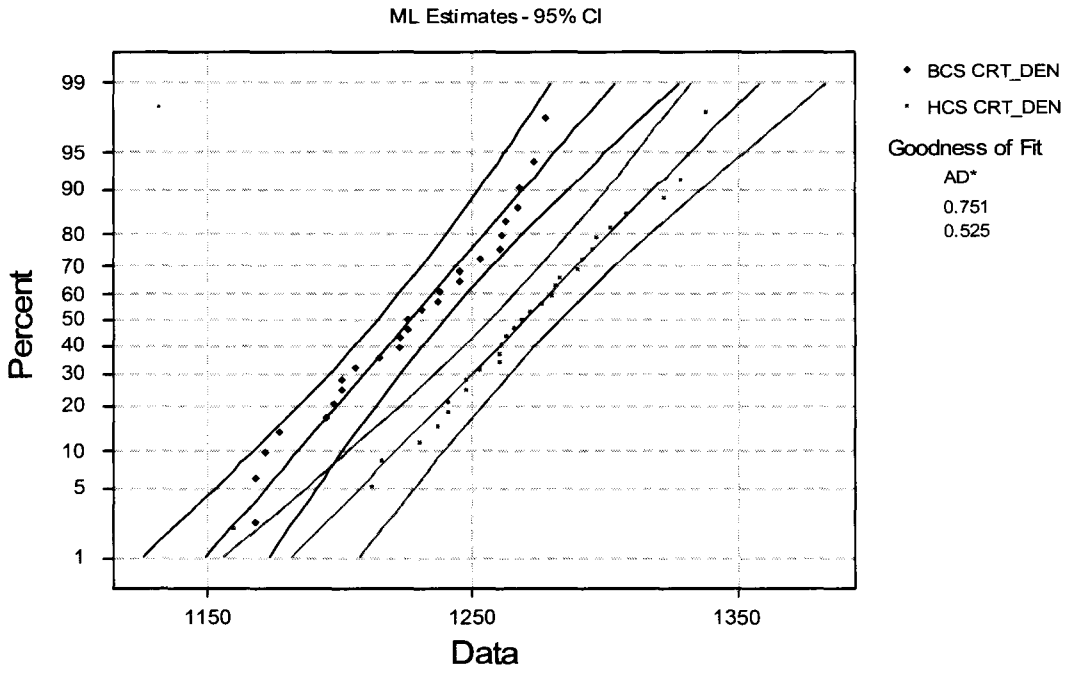


FIGURE 45: NORMAL PROBABILITY PLOT OF TOT_CNT AT THE 20% RADIUS

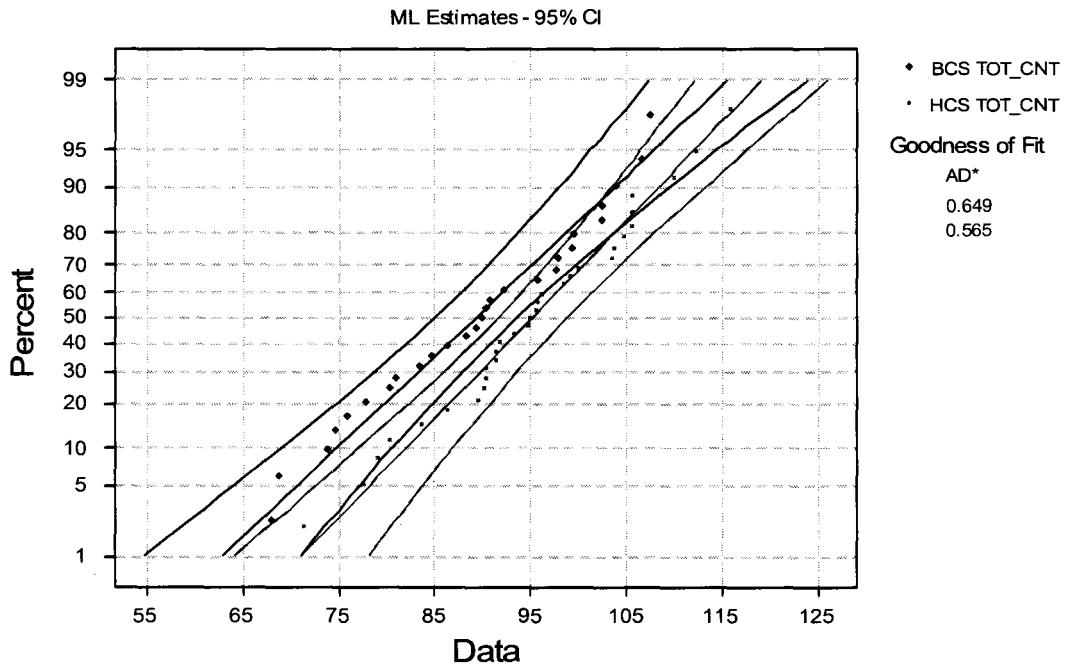


FIGURE 46: NORMAL PROBABILITY PLOT OF CRT_CNT AT THE 20% RADIUS

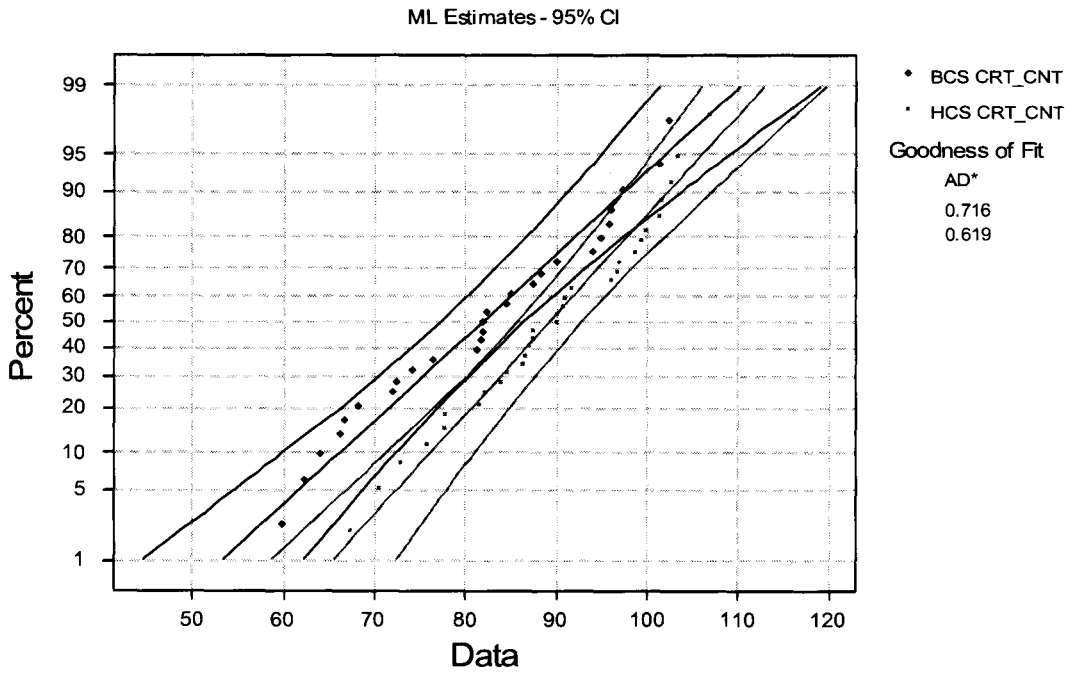


FIGURE 47: NORMAL PROBABILITY PLOT OF CRT_A AT THE 20% RADIUS

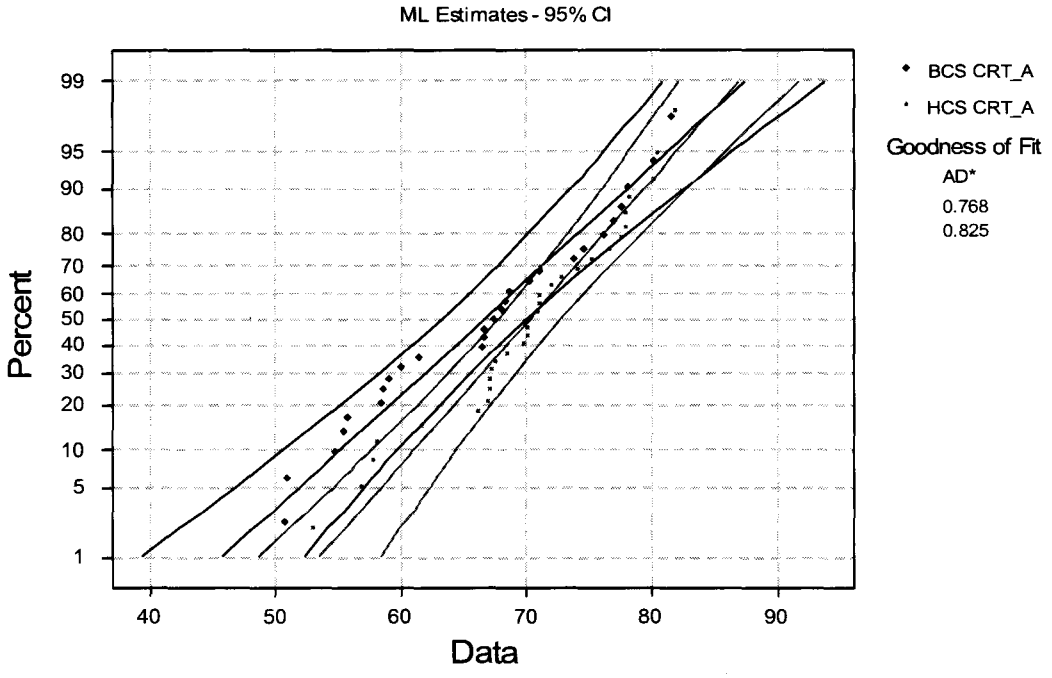


FIGURE 48: NORMAL PROBABILITY PLOT OF CRT_THK AT THE 20% RADIUS

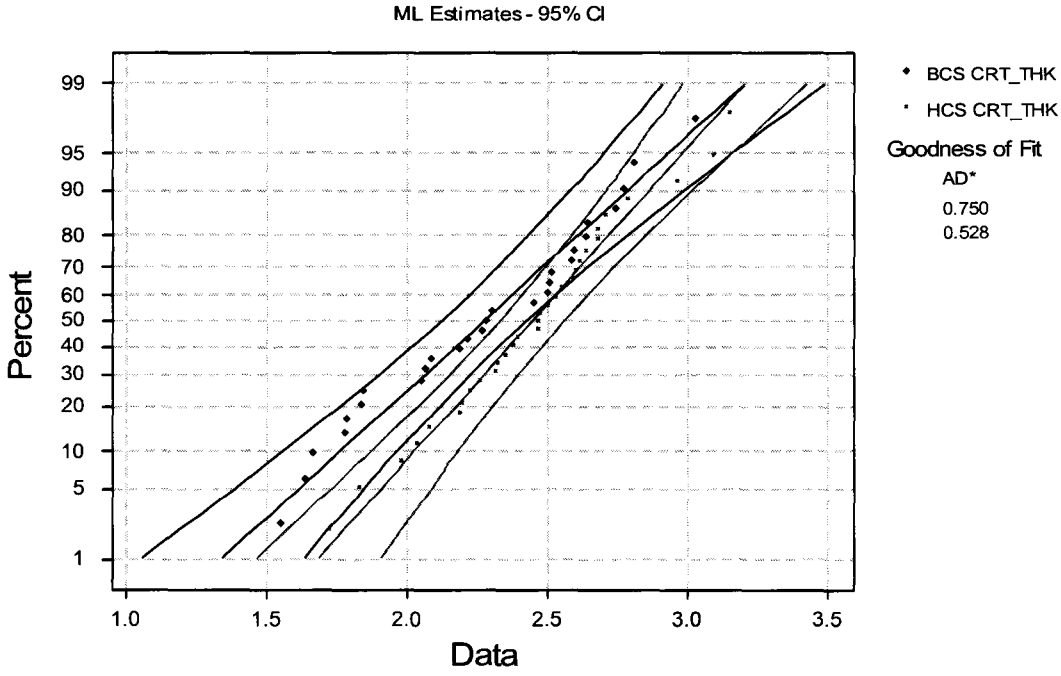


FIGURE 49: NORMAL PROBABILITY PLOT OF PERI_C AT THE 20% RADIUS

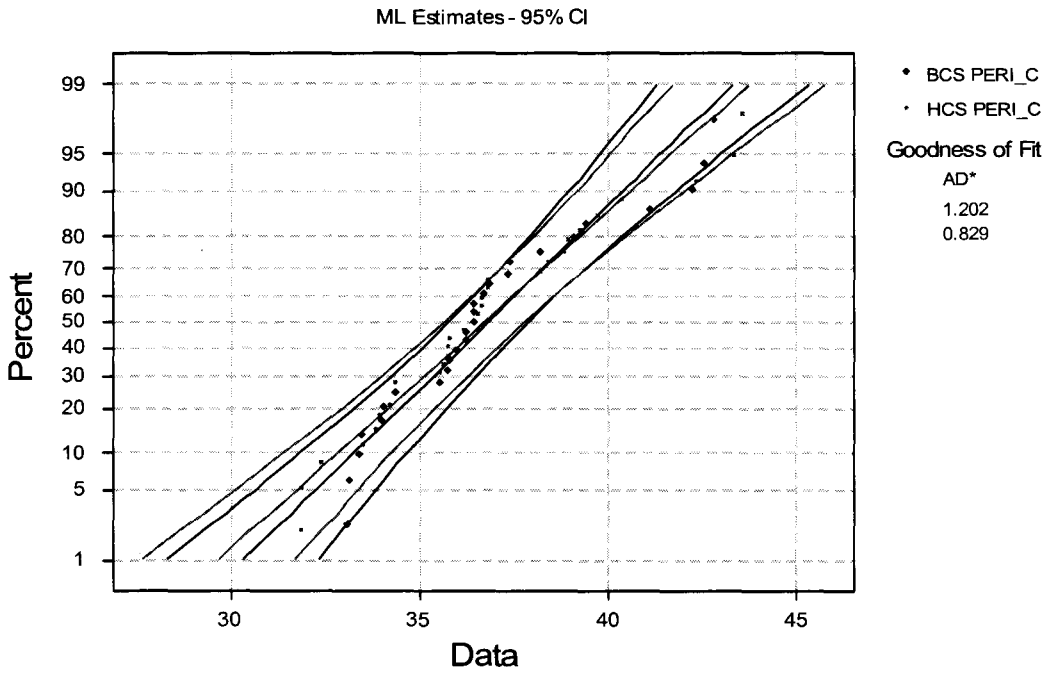


FIGURE 50: NORMAL PROBABILITY PLOT OF ENDO_C AT THE 20% RADIUS

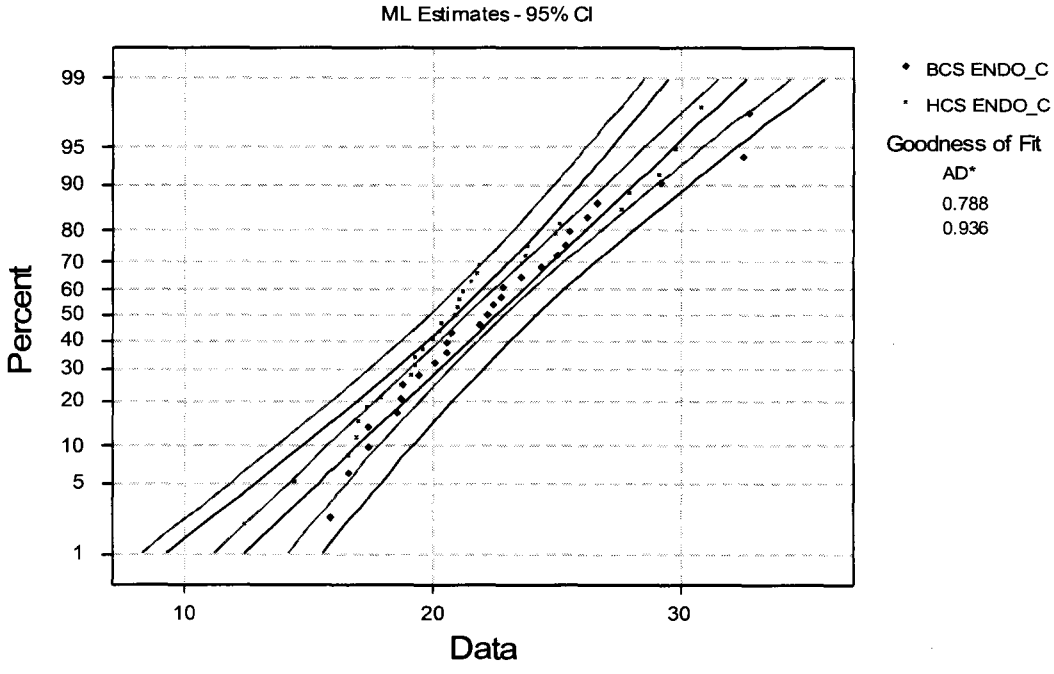


FIGURE 51: NORMAL PROBABILITY PLOT OF PMI AT THE 20% RADIUS

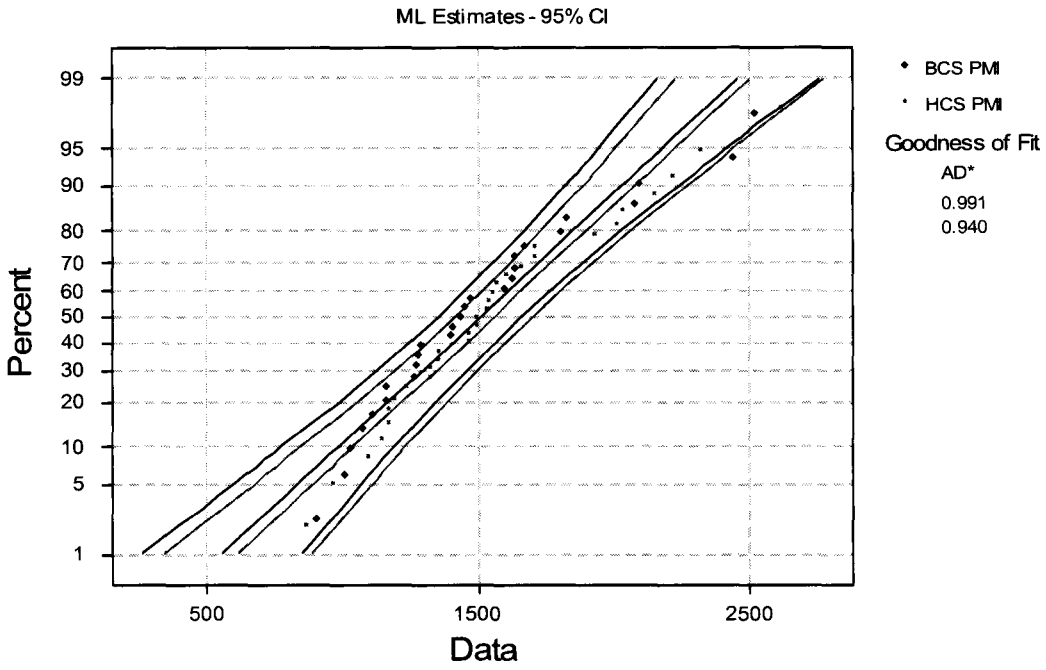


FIGURE 52: NORMAL PROBABILITY PLOT OF PMR AT THE 20% RADIUS

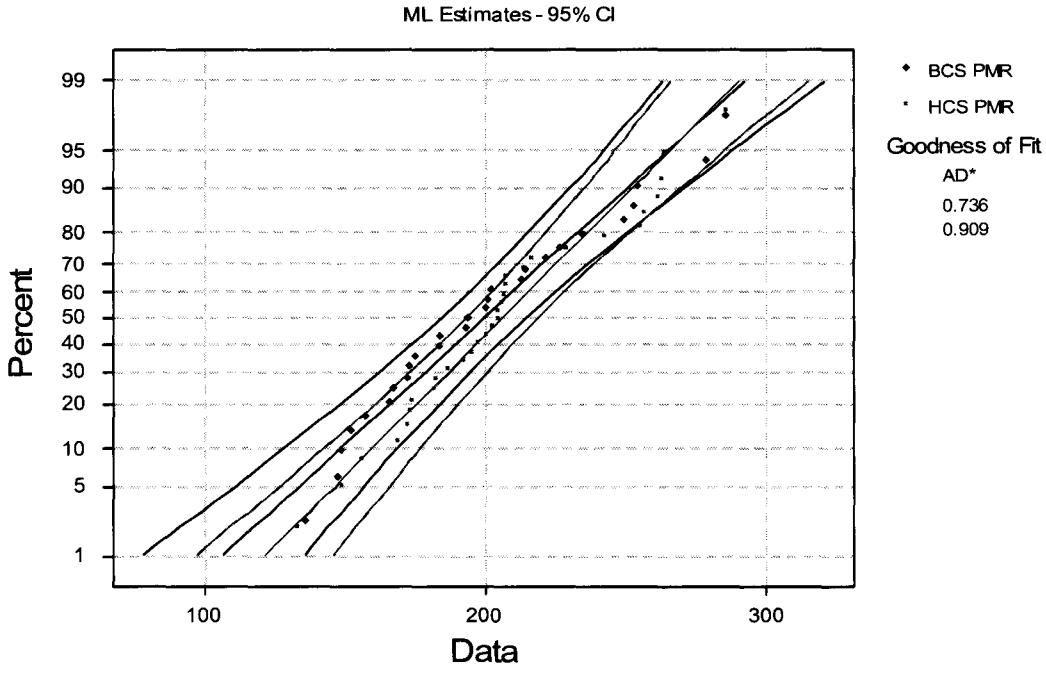


FIGURE 53: NORMAL PROBABILITY PLOT OF SSI AT THE 20% RADIUS

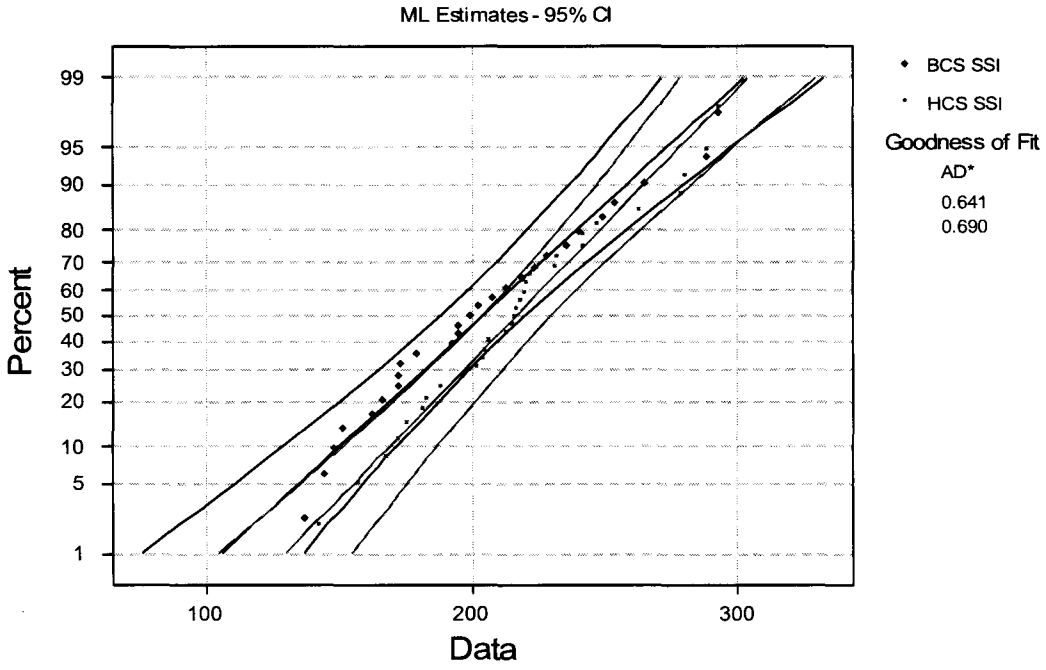


FIGURE 54: REGRESSION ANALYSIS OF CRT_DEN AND BMI AT THE 20% RADIUS

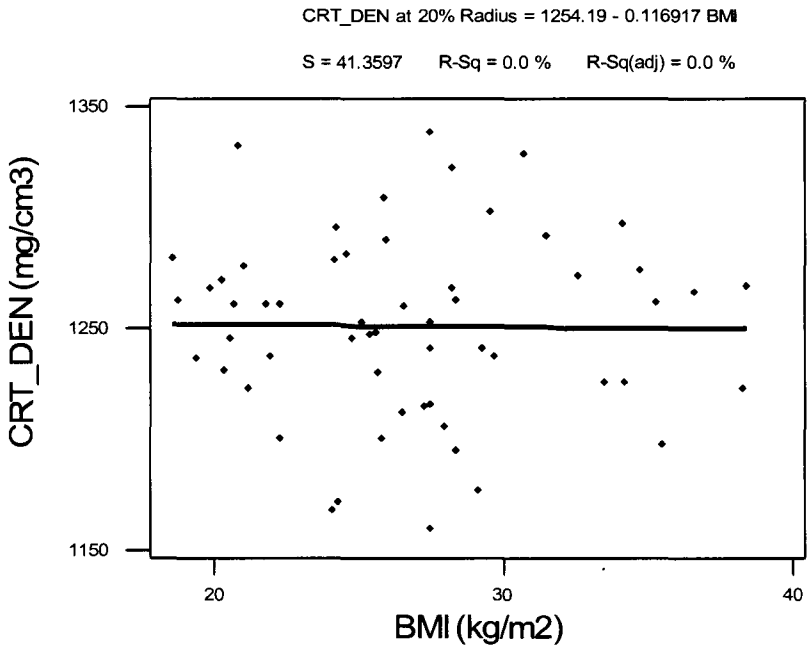


FIGURE 55: REGRESSION ANALYSIS OF TOT_CNT AND BMI AT THE 20% RADIUS

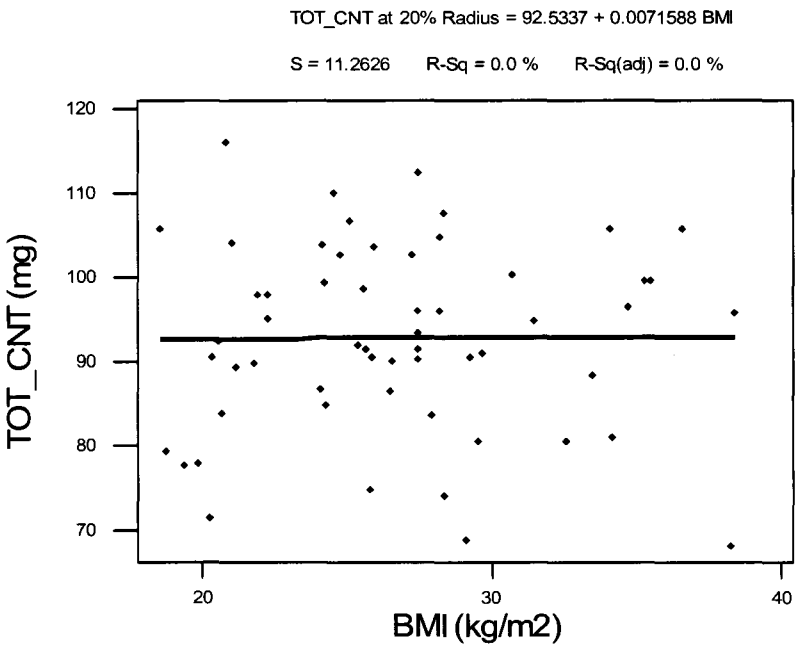


FIGURE 56: REGRESSION ANALYSIS OF CRT_CNT AND BMI AT THE 20% RADIUS

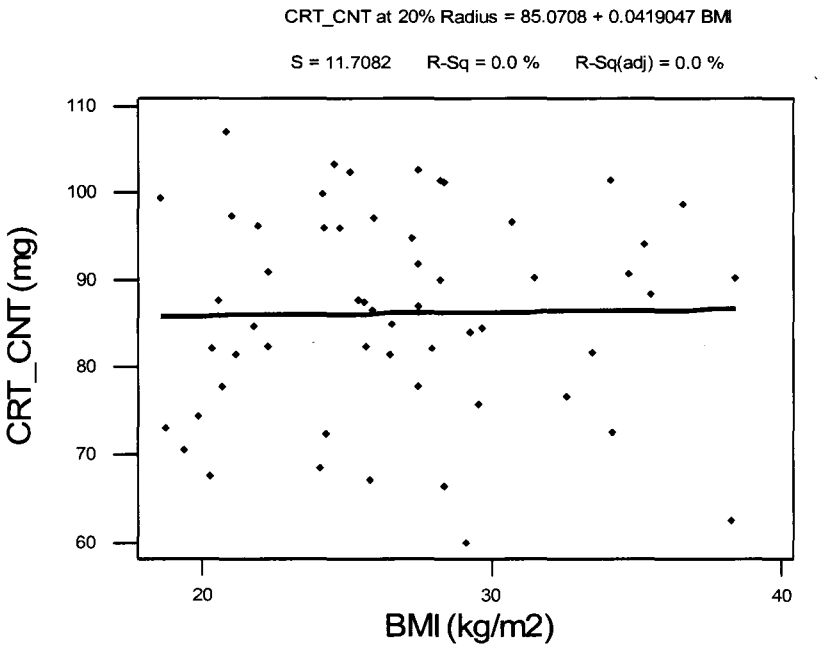


FIGURE 57: REGRESSION ANALYSIS OF CRT_DEN AND AGE AT THE 20% RADIUS

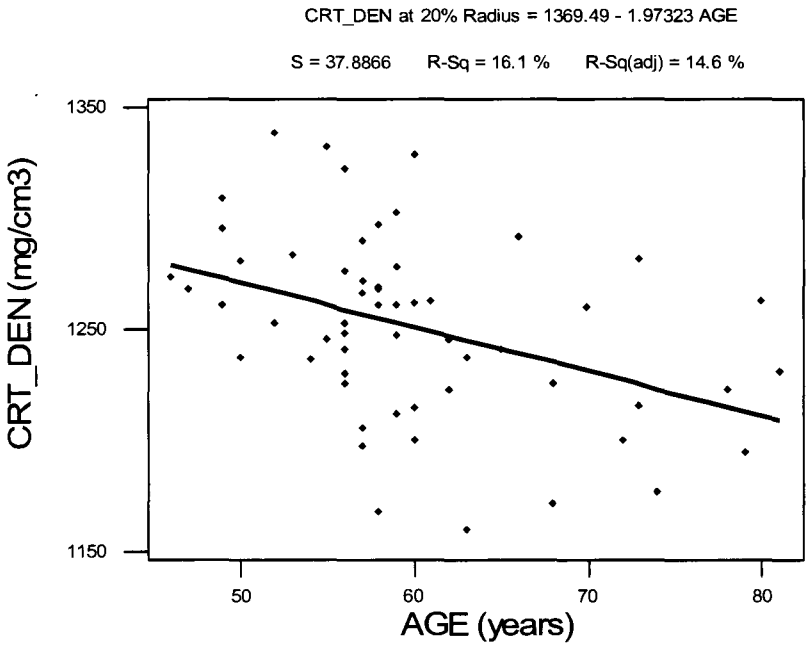


FIGURE 58: REGRESSION ANALYSIS OF TOT_CNT AND AGE AT THE 20% RADIUS

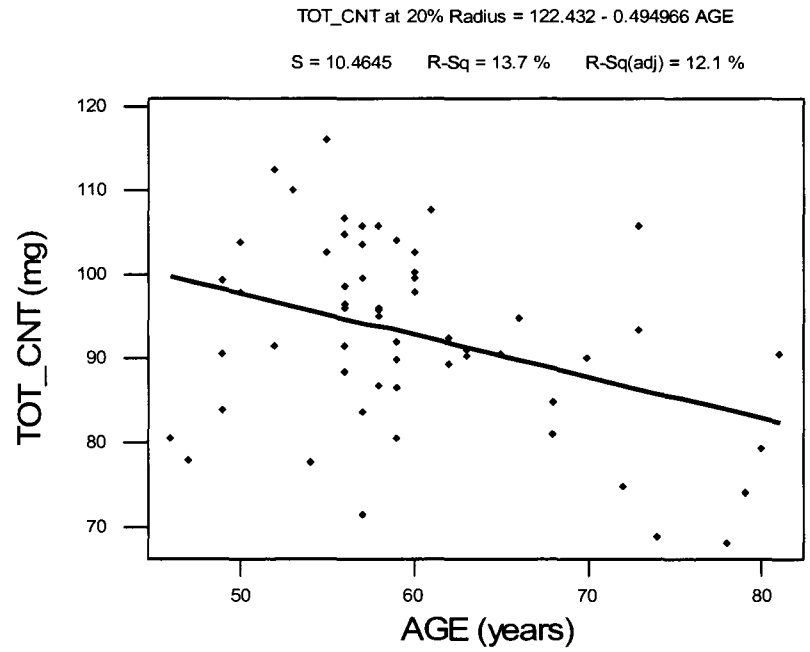


FIGURE 59: REGRESSION ANALYSIS OF CRT_CNT AND AGE AT THE 20% RADIUS

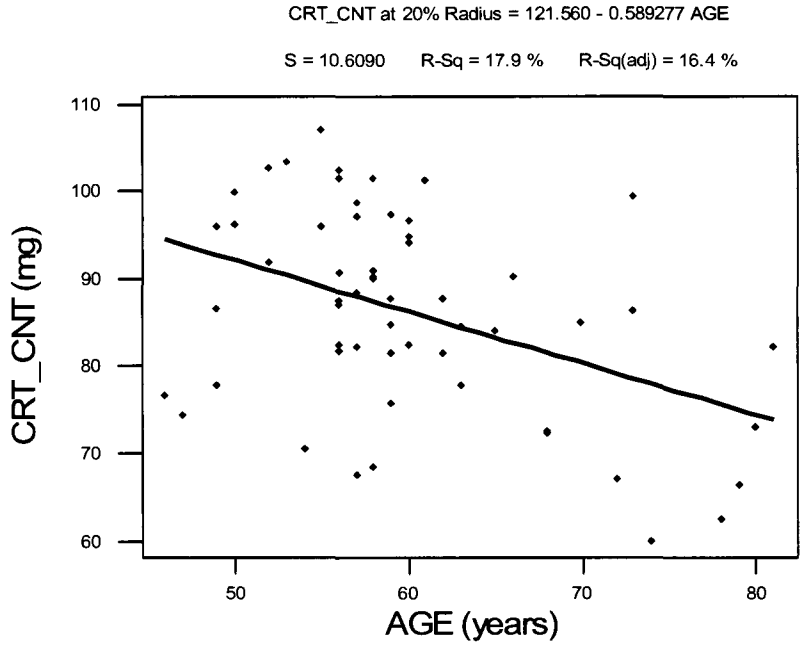


FIGURE 60: REGRESSION ANALYSIS OF CRT_DEN AND YSM AT THE 20% RADIUS

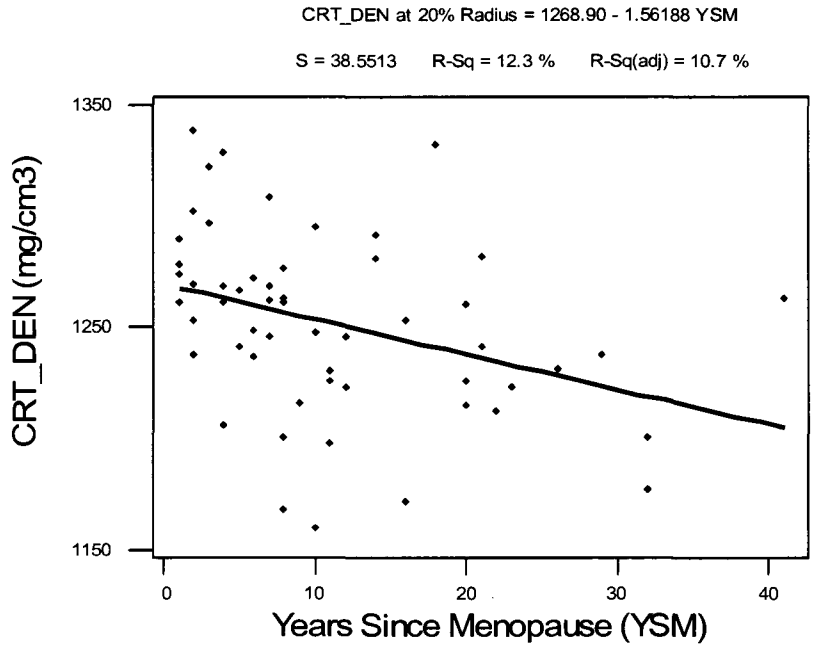


FIGURE 61: REGRESSION ANALYSIS OF TOT_CNT AND YSM AT THE 20% RADIUS

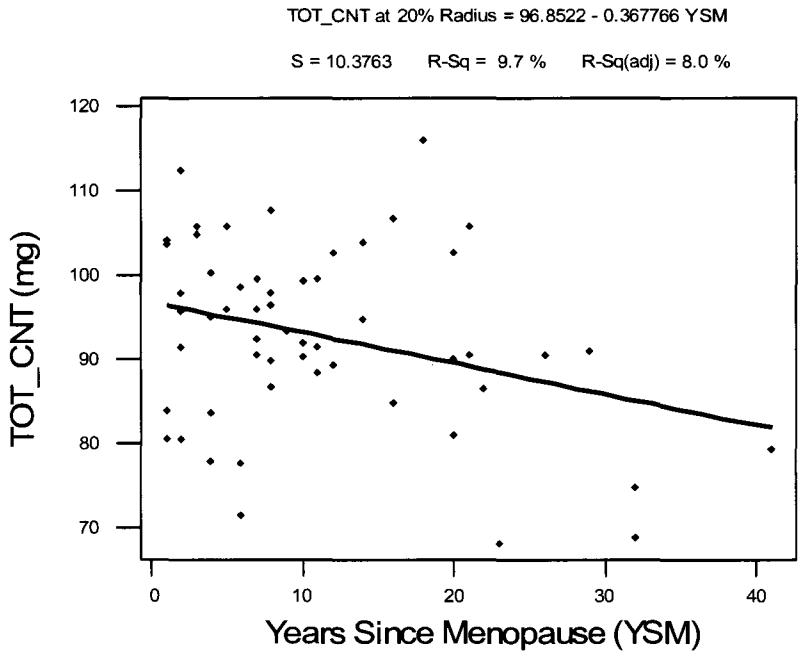


FIGURE 62: REGRESSION ANALYSIS OF CRT_CNT AND YSM AT THE 20% RADIUS

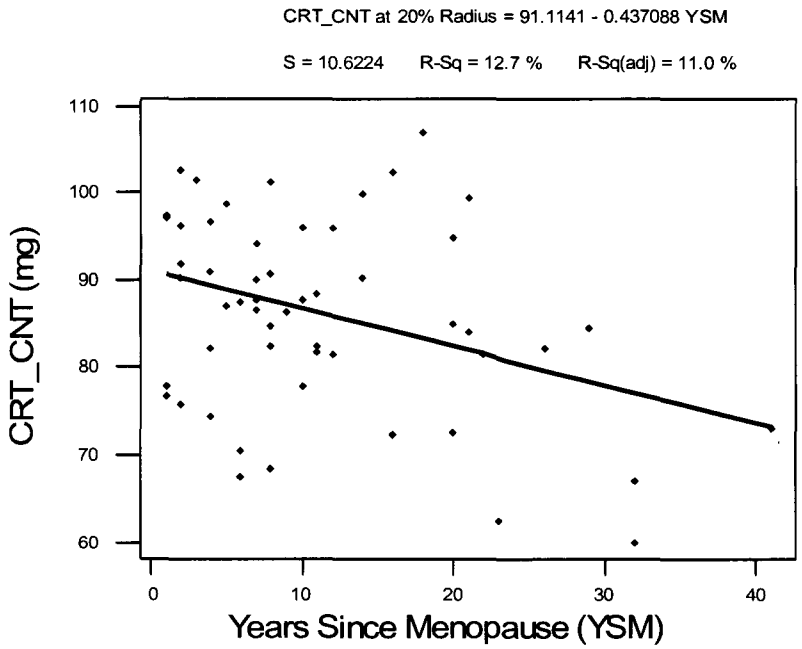


FIGURE 63: REGRESSION ANALYSIS OF CRT_DEN AND AAM AT THE 20% RADIUS

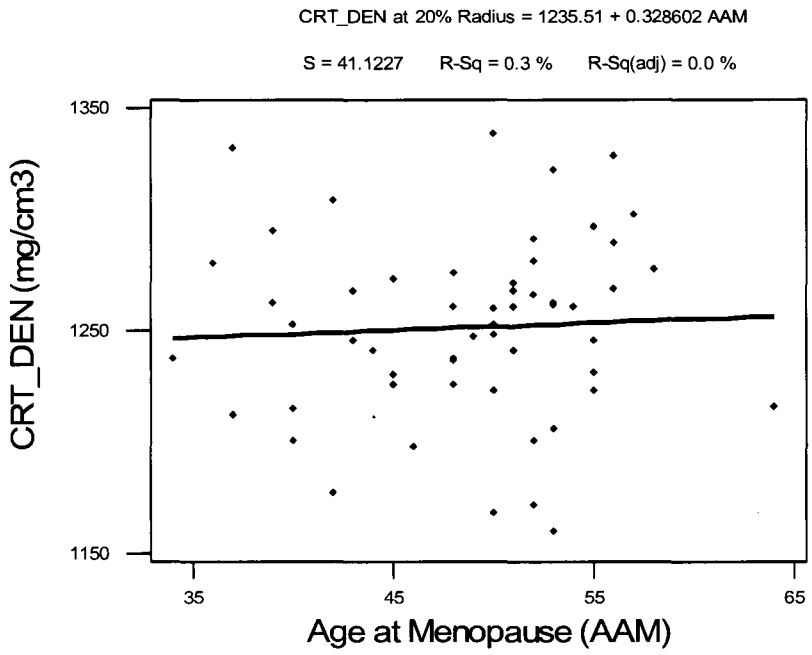


FIGURE 64: REGRESSION ANALYSIS OF TOT_CNT AND AAM AT THE 20% RADIUS

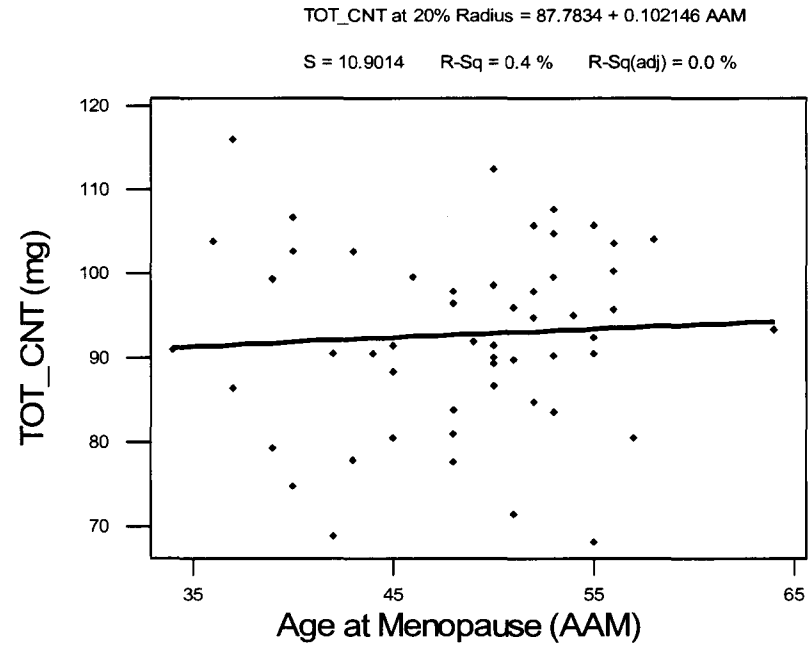


FIGURE 65: REGRESSION ANALYSIS OF CRT_CNT AND AAM AT THE 20% RADIUS

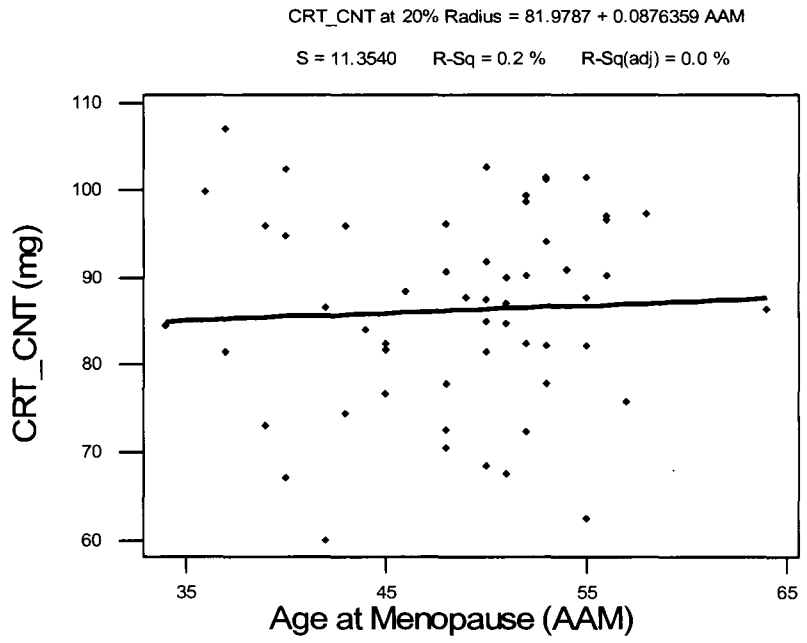


FIGURE 66: REGRESSION ANALYSIS OF CRT_DEN AND NOC AT THE 20% RADIUS

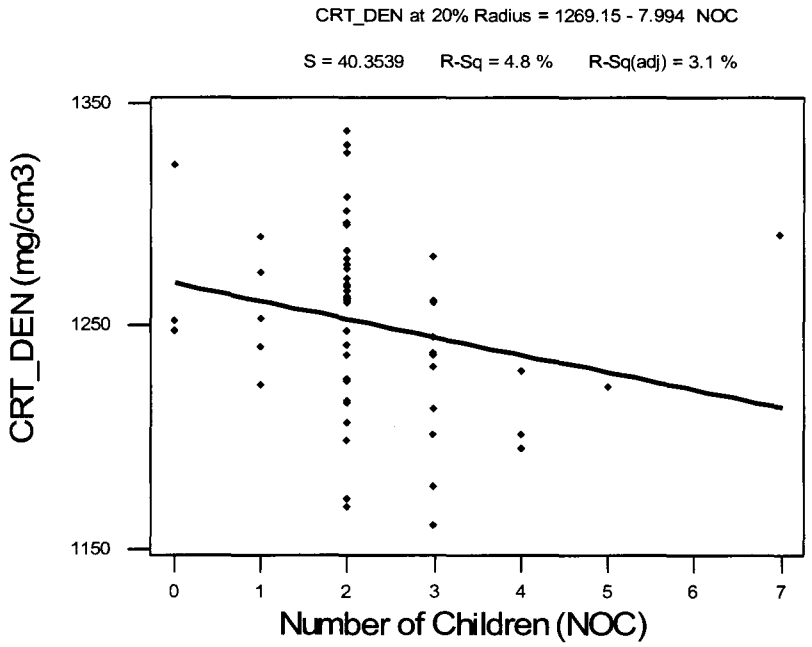


FIGURE 67: REGRESSION ANALYSIS OF TOT_CNT AND NOC AT THE 20% RADIUS

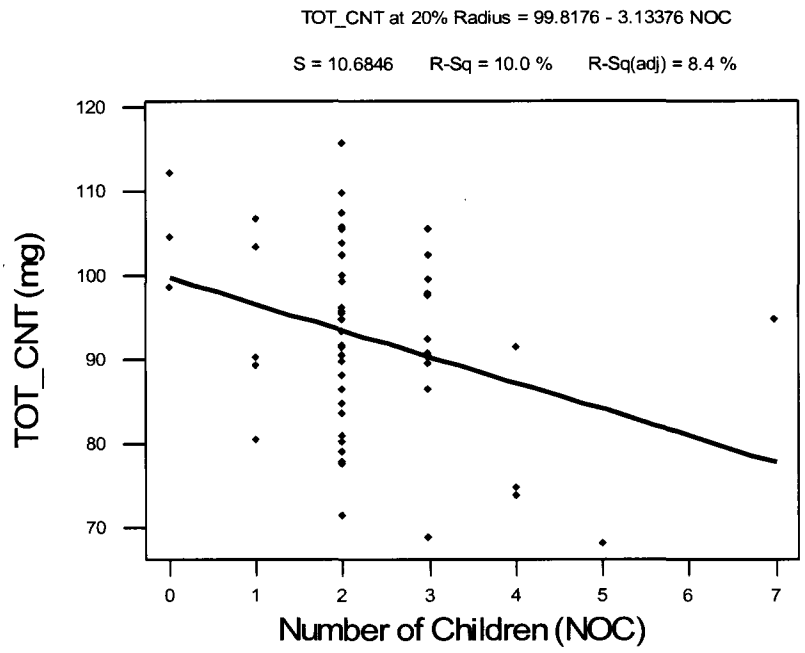


FIGURE 68: REGRESSION ANALYSIS OF CRT_CNT AND NOC AT THE 20% RADIUS

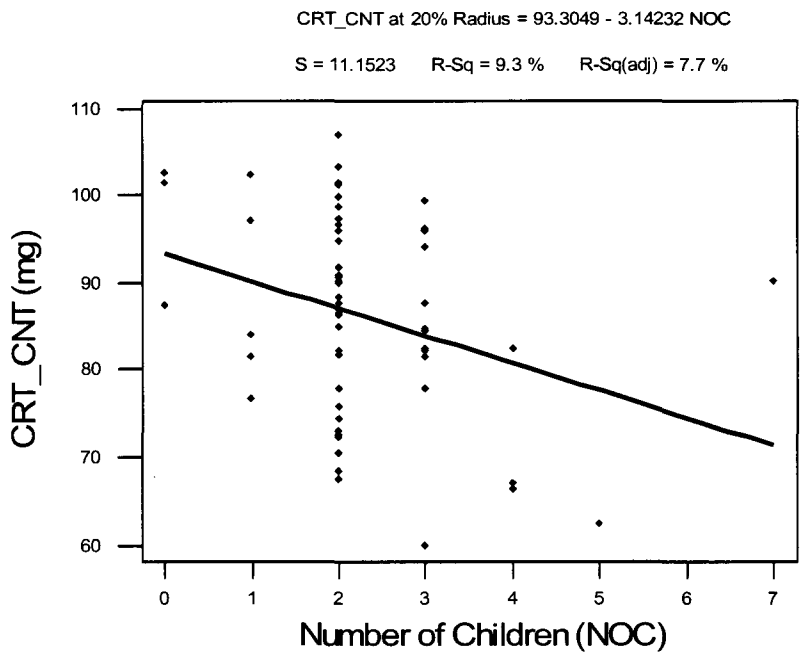


FIGURE 69: REGRESSION ANALYSIS OF CRT_DEN AND TOA AT THE 20% RADIUS

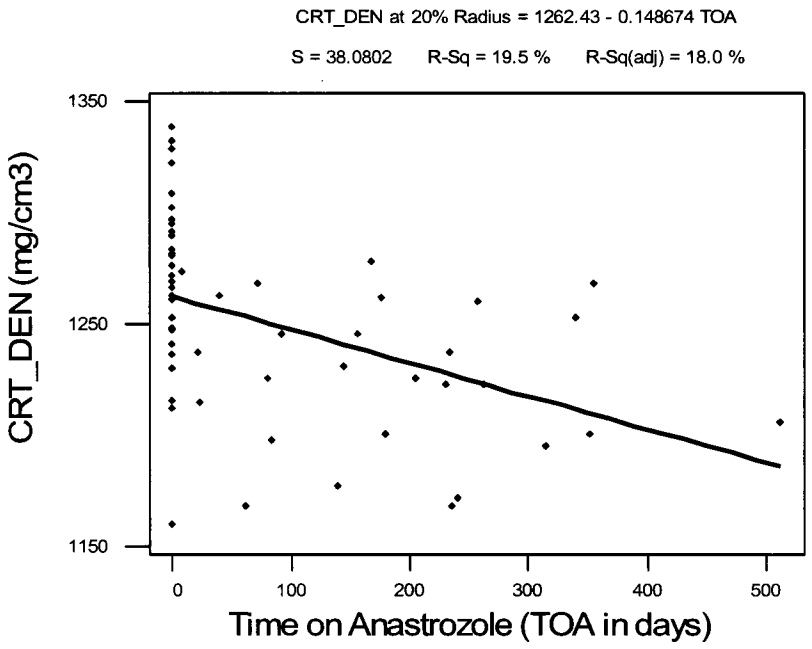


FIGURE 70: REGRESSION ANALYSIS OF TOT_CNT AND TOA AT THE 20% RADIUS

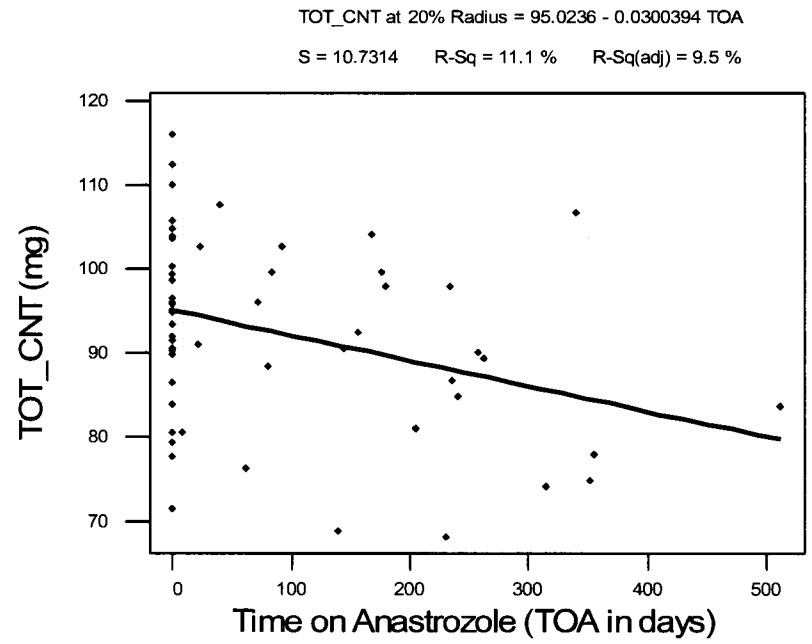
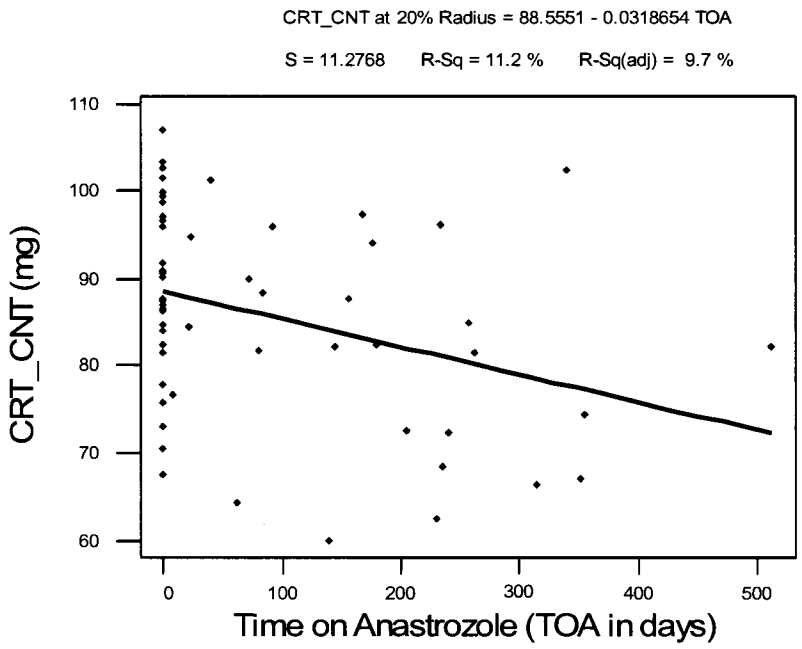


FIGURE 71: REGRESSION ANALYSIS OF CRT_CNT AND TOA AT THE 20% RADIUS



4.3.4 Diaphyseal Tibia

Table 18 displays the descriptive statistics of pQCT derived density, mass, area, and geometry at the 38% diaphyseal site of the non-dominant tibia. Scans were performed on the non-dominant tibia of each subject using the standard imaging procedure as described previously in section *3.3*. Also given in the table, where applicable, is the statistical significance of the difference between the measured parameters in the healthy and breast cancer subjects. One scan at the 38% site of a breast cancer subject displayed an inadequately high level of motion artifact; this scan was excluded from further analysis for this reason. Therefore, at the 38% tibia site, the sample size of breast cancer subjects was n=26.

TABLE 18: BONE AND GEOMETRIC VARIABLES AT THE 38% TIBIA

Variable[a]	All Subjects (n = 57)	Healthy Post-Menopausal Subjects (n = 31)	Breast Cancer Subjects (n = 26)
Density (mg/cm^3)			
TOT_DEN	846 (13.2)	852 (19.7)	838 (17.4)
CRT_DEN	1192 (4.5)	1209 (5.2)	1173 (5.6)**
Mass (mg)			
TOT_CNT	333 (4.6)	340 (6.6)	326 (6.1)
CRT_CNT	310 (4.4)	317 (5.7)	301 (6.3)
Area (mm^2)			
CRT_A	260 (3.5)	262 (4.5)	257 (5.5)
Geometry			
CRT_THK *(mm)*	4.7 (0.1)	4.7 (0.1)	4.7 (0.1)
PERI_CIR *(mm)*	71 (0.8)	71 (1.3)	70 (0.9)
ENDO_CIR *(mm)*	41 (1.2)	42 (1.9)	41 (1.4)
PMI *(mm^3)*	20274 (516)	20570 (726)	19921 (738)
PMR *(mm^3)*	1394 (25)	1414 (37)	1370 (34)
SSI *(mm^3)*	1383 (26)	1421 (37)	1337 (33)

[a]Data expressed as the mean (SEM); Abbreviations: TOT_DEN, total density; CRT_DEN, cortical density; TOT_CNT, total content; CRT_CNT, cortical content; TOT_A, total area; CRT-A, cortical area; CRT-THK, cortical thickness; ENDO_CIR, endosteal circumference; PERI_CIR, periosteal circumference; PMI; polar moment of inertia; PMR, polar moment of resistance; SSI, stress-strain index; **p< 0.01 for 2 sample t-test

At this site, only the CRT_DEN values showed a significantly lower ($p<0.01$) result among breast cancer subjects. The mean CRT_DEN was measured as 1209 mg/cm^3 in the control subjects and 1173 mg/cm^3 in the breast cancer subjects. The mean values for TOT_DEN were 852 mg/cm^3 and 838 mg/cm^3 for each group respectively. The mean values for total and cortical mass were also somewhat lower in the breast cancer subjects, as were the cortical area and all geometry parameters except CRT_THK, which was the same for both groups at 4.7 (0.1) mm. However, these differences did not achieve statistical significance. Normality of the data sets was assessed, and the normal distribution plots, the 95% confidence intervals for all the variables measured at the diaphyseal radius, as well as the data distribution for both

cohorts of subjects, are displayed for each pQCT variable in Figures 72 through 82. Analysis was conducted to determine any relationship between CRT_DEN and the anthropometric characteristics of the study sample. At the 38% tibia, univariate regression analysis demonstrate that less than 6% of the variation in CRT_DEN can be accounted for by the age of the study participants (R^2=5%) and the YSM (R^2=5.5%). The variation due to BMI (R^2=0.2%), AAM (R^2=0.4%), and NOC (R^2=2.8%) were all very low. TOA contributed to the highest variation in the data set (R^2=15.1%). The regression analysis, including the regression equation and R^2 values for the measured pQCT variables at the 20% diaphyseal radius site are shown in Figures 83 through 88.

FIGURE 72: NORMAL PROBABILITY PLOT OF TOT_DEN AT THE 38% TIBIA

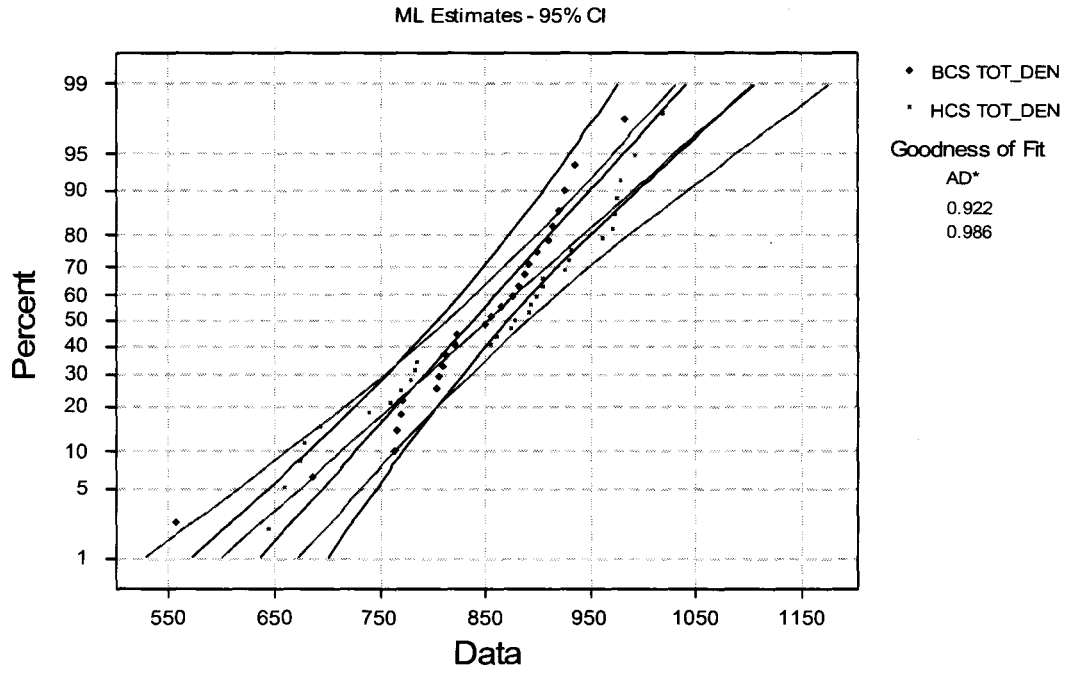


FIGURE 73: NORMAL PROBABILITY PLOT OF CRT_DEN AT THE 38% TIBIA

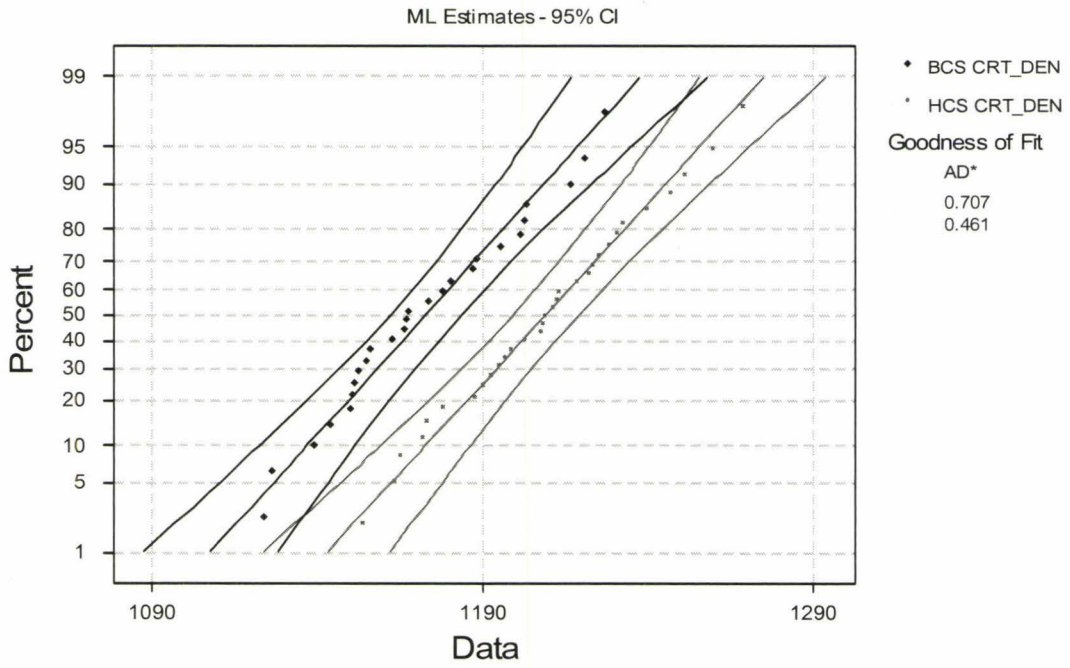


FIGURE 74: NORMAL PROBABILITY PLOT OF TOT_CNT AT THE 38% TIBIA

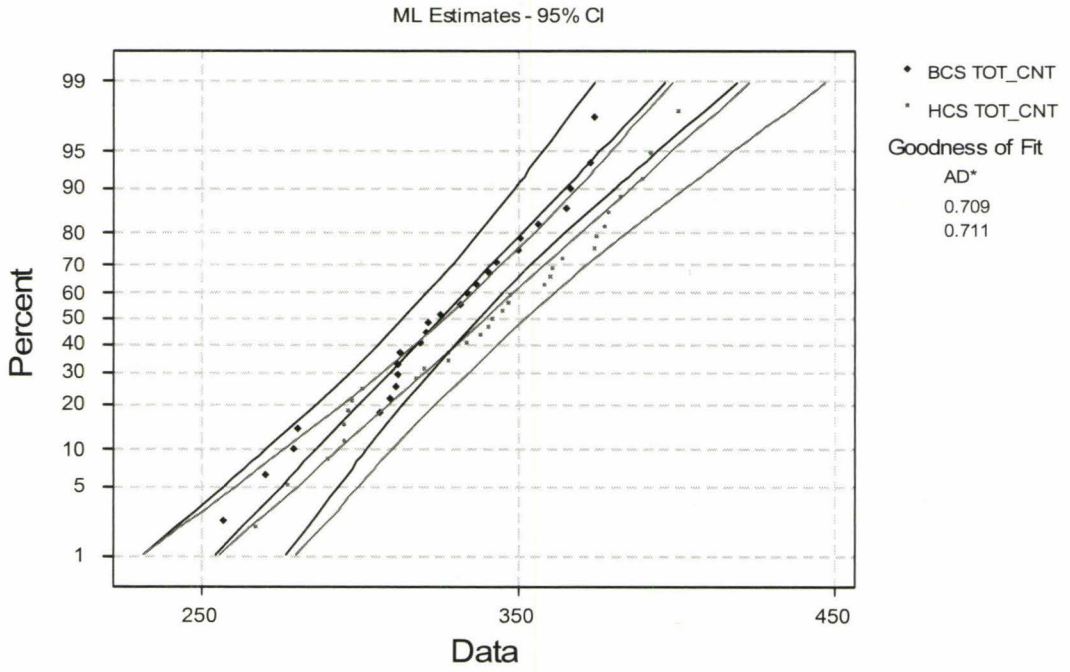


FIGURE 75: NORMAL PROBABILITY PLOT OF CRT_CNT AT THE 38% TIBIA

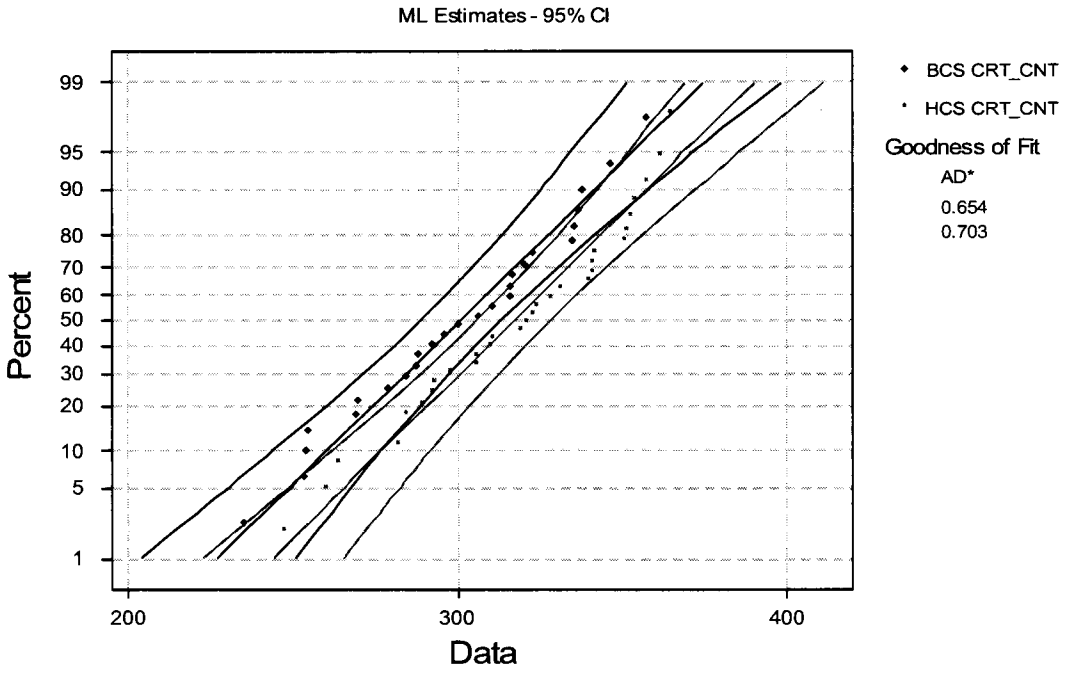


FIGURE 76: NORMAL PROBABILITY PLOT OF CRT_A AT THE 38% TIBIA

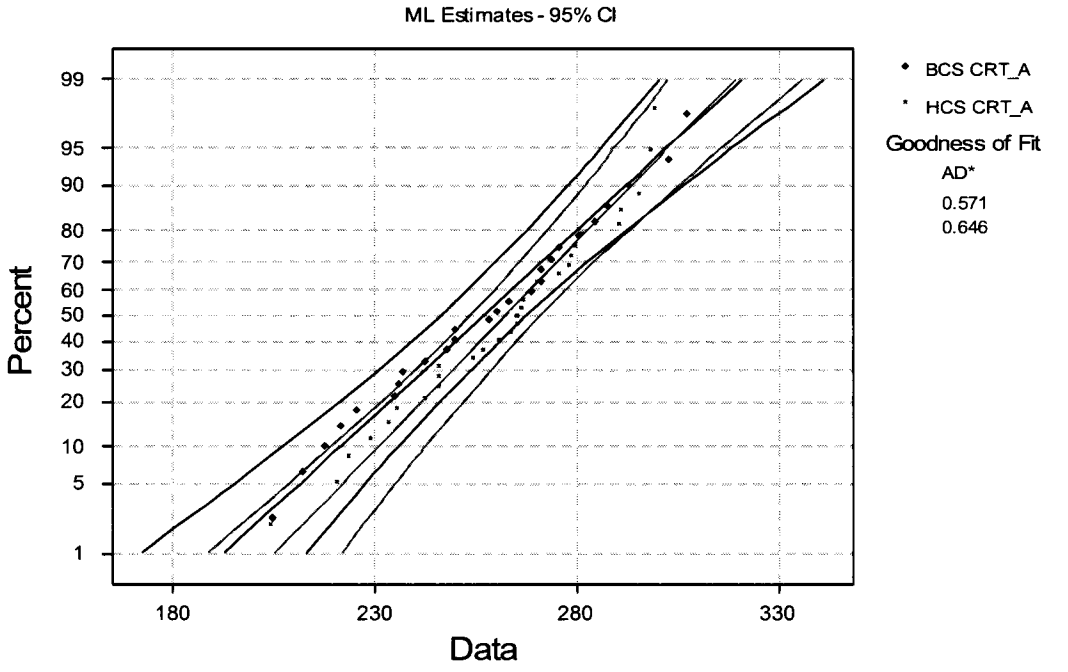


FIGURE 77: NORMAL PROBABILITY PLOT OF CRT_THK AT THE 38% TIBIA

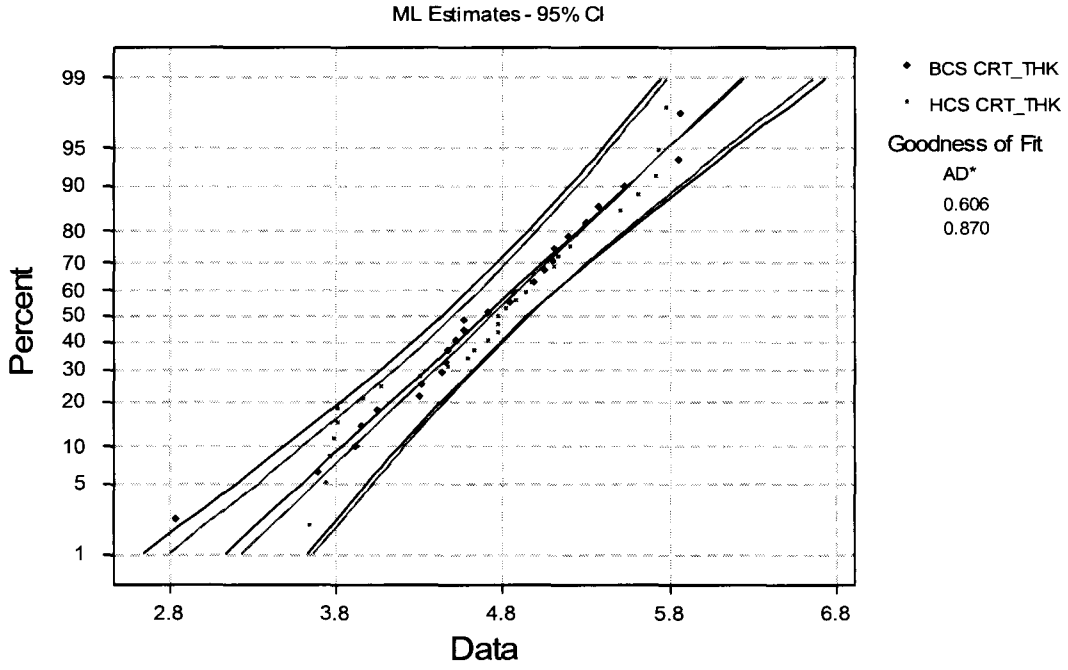


FIGURE 78: NORMAL PROBABILITY PLOT OF PERI_C AT THE 38% TIBIA

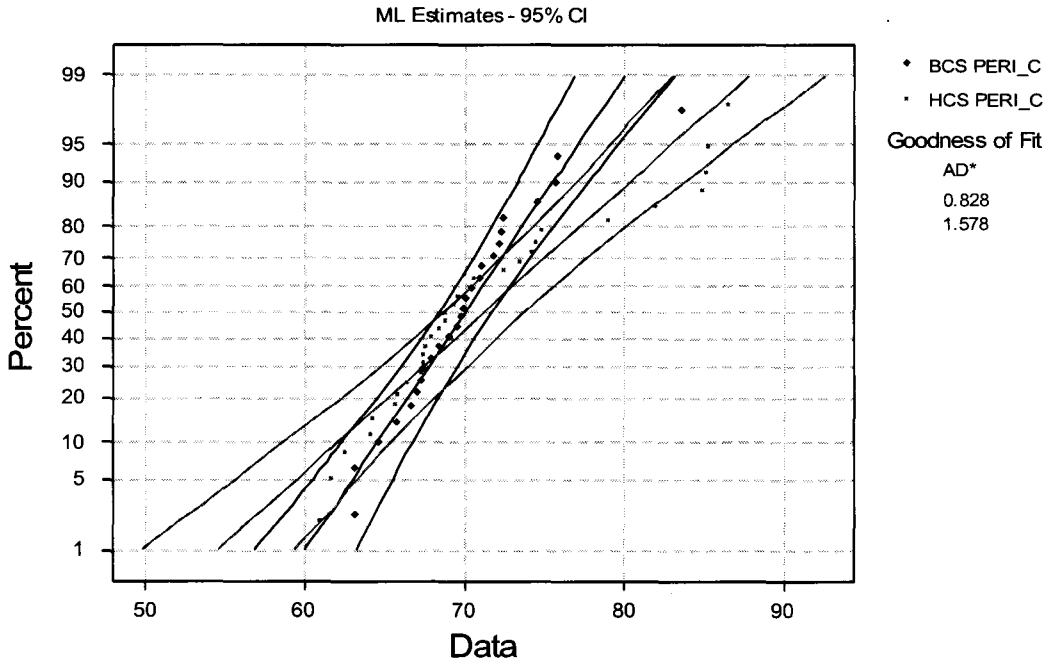


FIGURE 79: NORMAL PROBABILITY PLOT OF ENDO_C AT THE 38% TIBIA

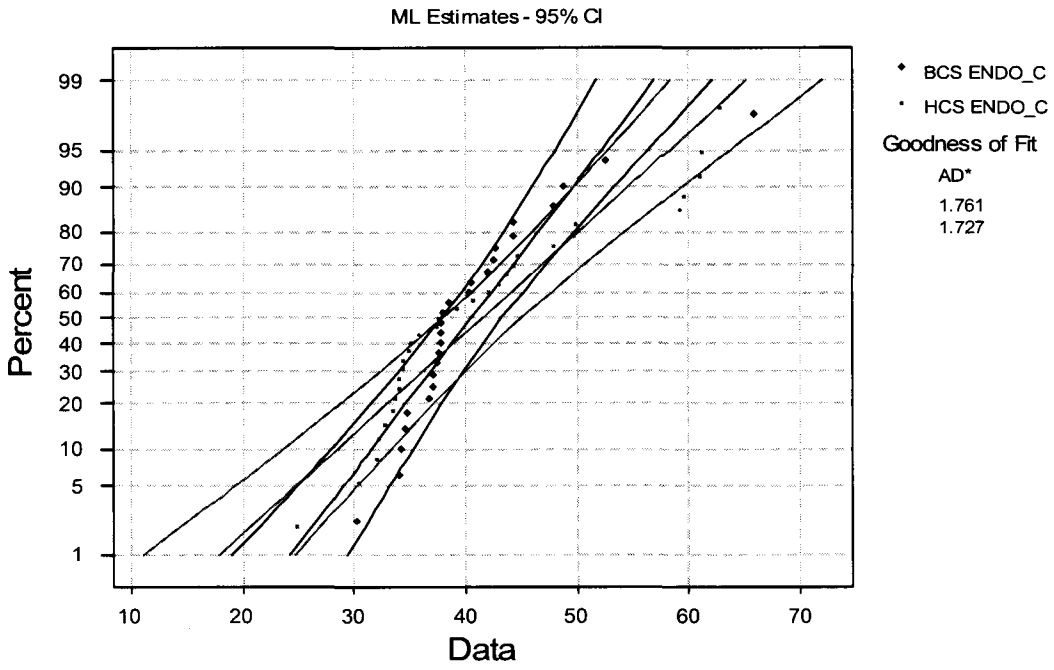


FIGURE 80: NORMAL PROBABILITY PLOT OF PMI AT THE 38% TIBIA

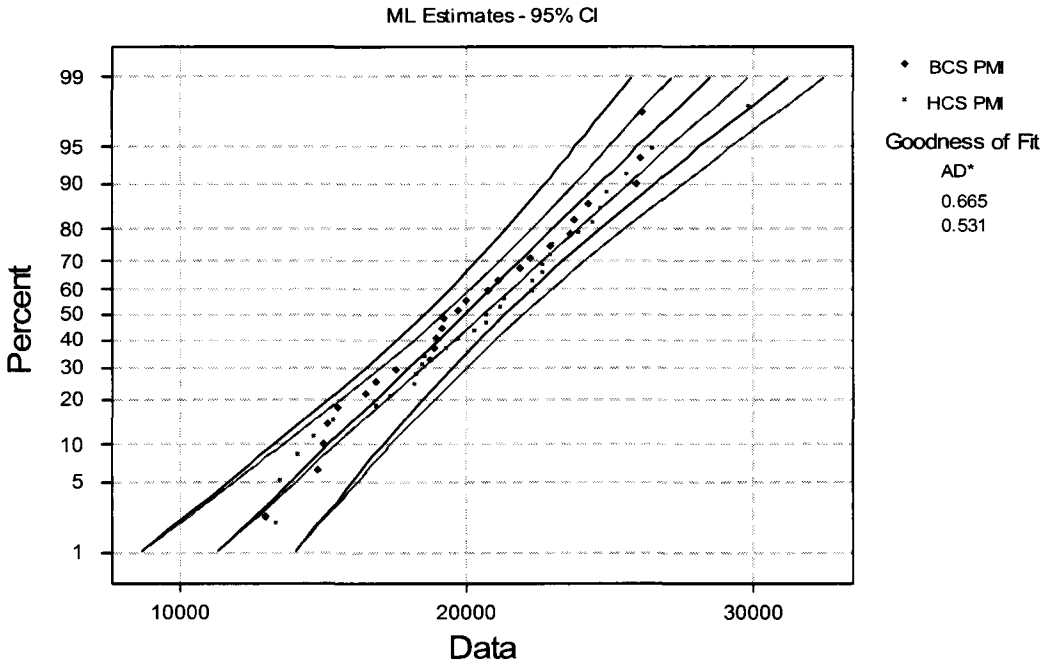


FIGURE 81: NORMAL PROBABILITY PLOT OF PMR AT THE 38% TIBIA

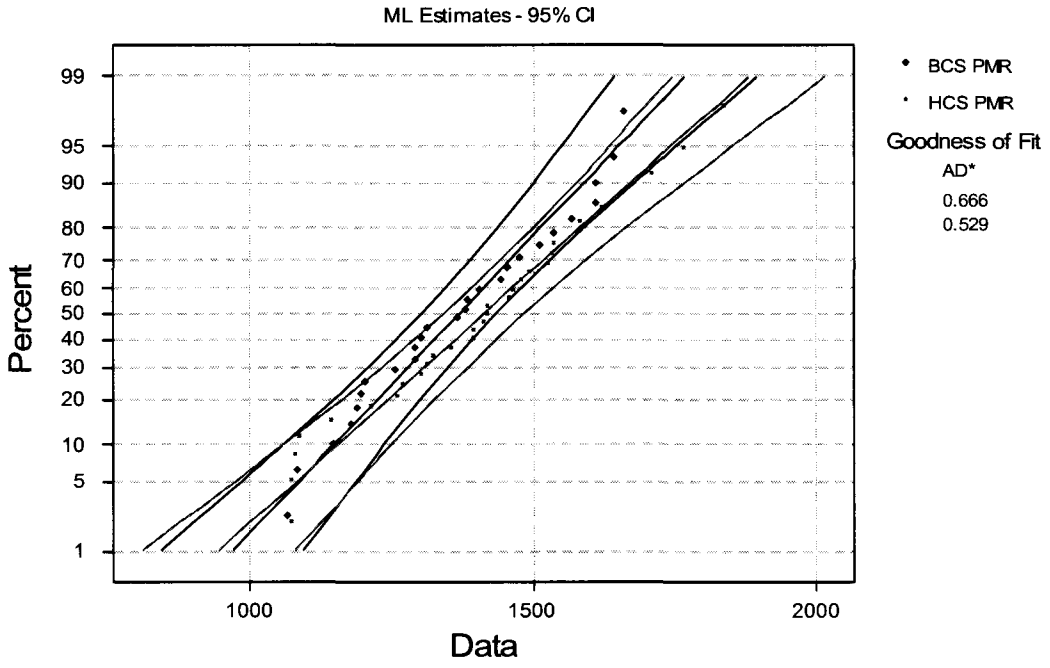


FIGURE 82: NORMAL PROBABILITY PLOT OF SSI AT THE 38% TIBIA

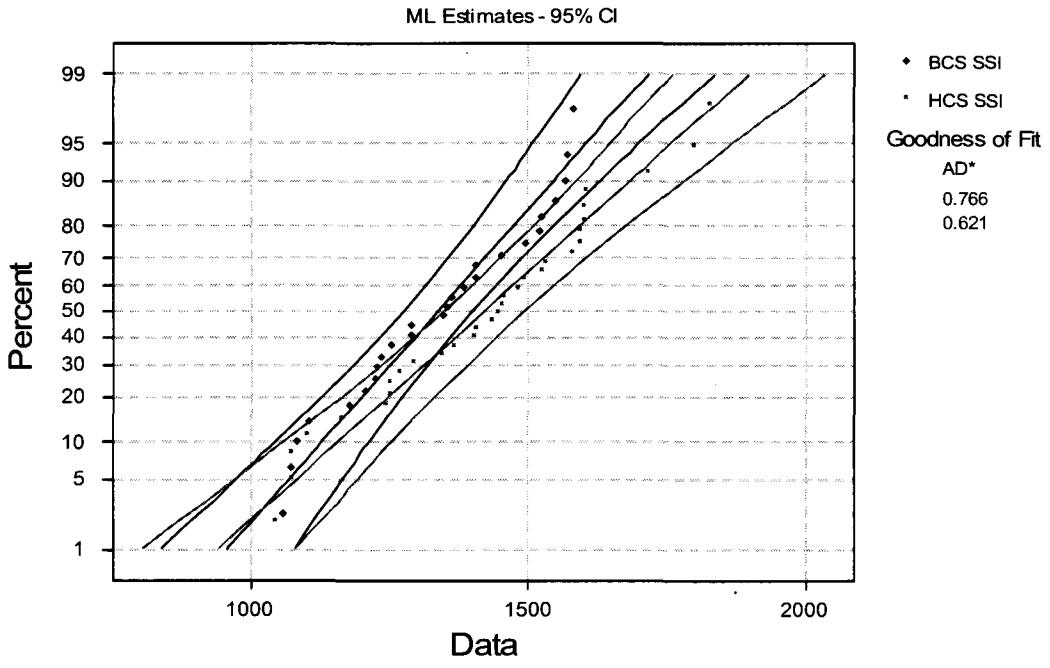


FIGURE 83: REGRESSION ANALYSIS OF CRT_DEN AND BMI AT THE 38% TIBIA

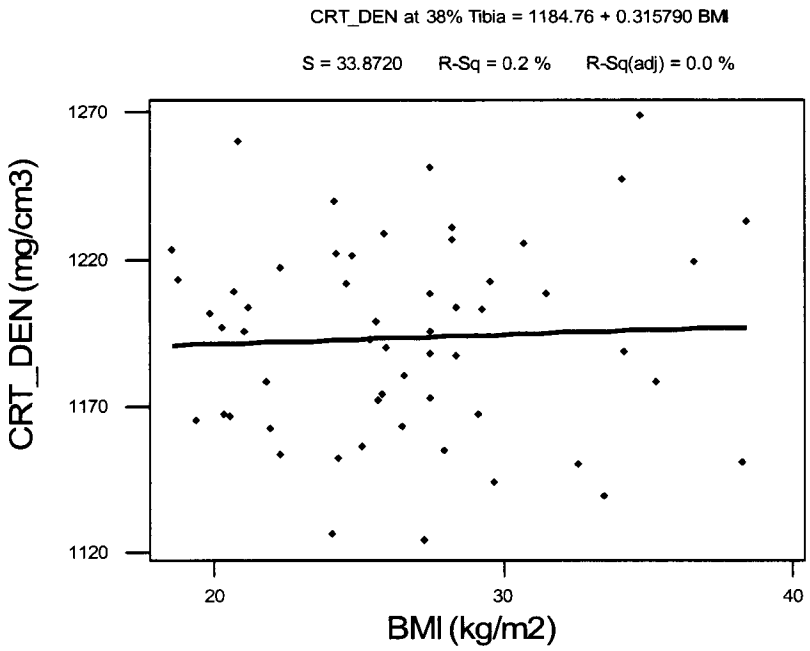


FIGURE 84: REGRESSION ANALYSIS OF CRT_DEN AND AGE AT THE 38% TIBIA

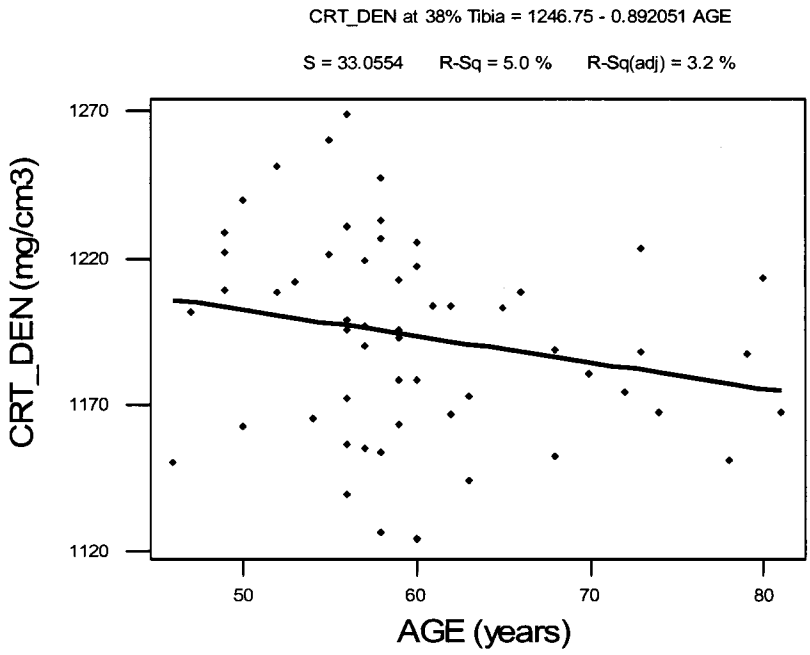


FIGURE 85: REGRESSION ANALYSIS OF CRT_DEN AND AAM AT THE 38% TIBIA

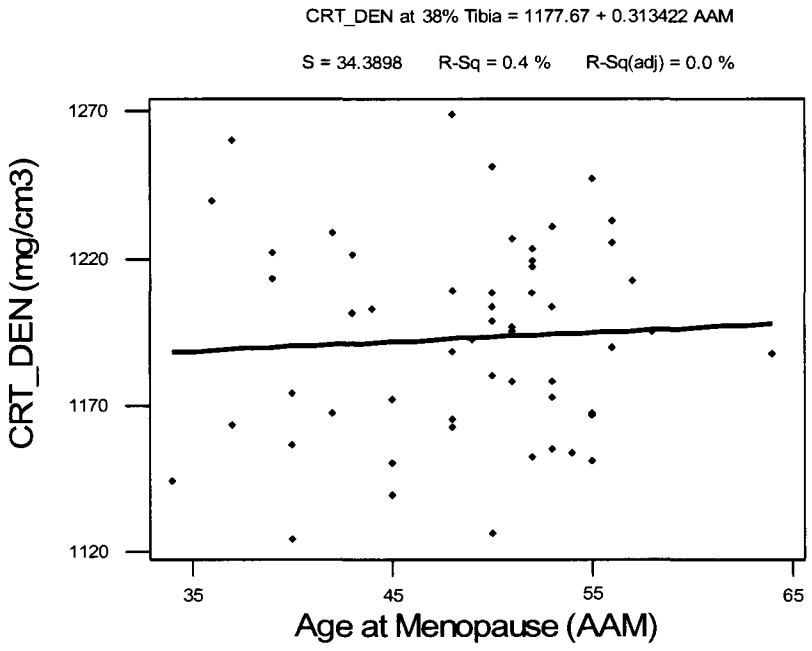


FIGURE 86: REGRESSION ANALYSIS OF CRT_DEN AND YSM AT THE 38% TIBIA

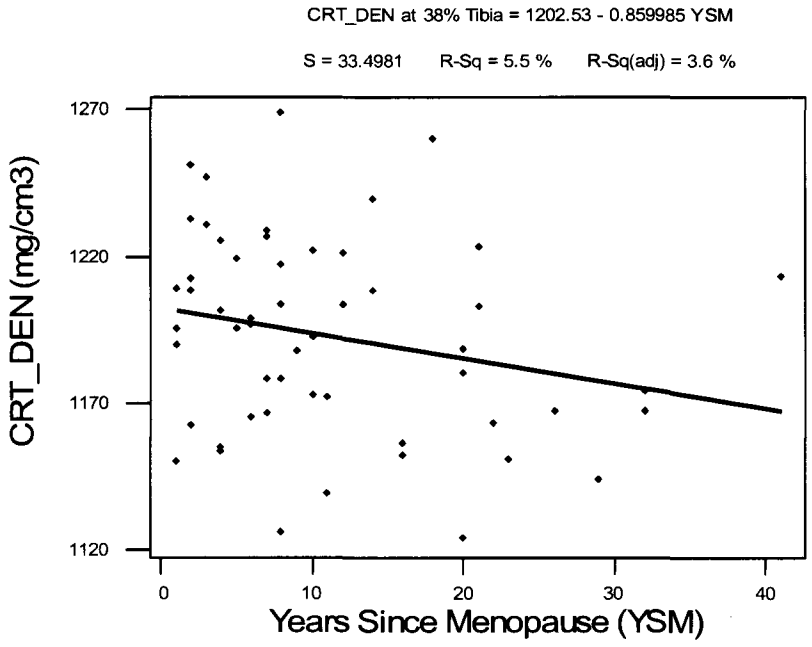


FIGURE 87: REGRESSION ANALYSIS OF CRT_DEN AND NOC AT THE 38% TIBIA

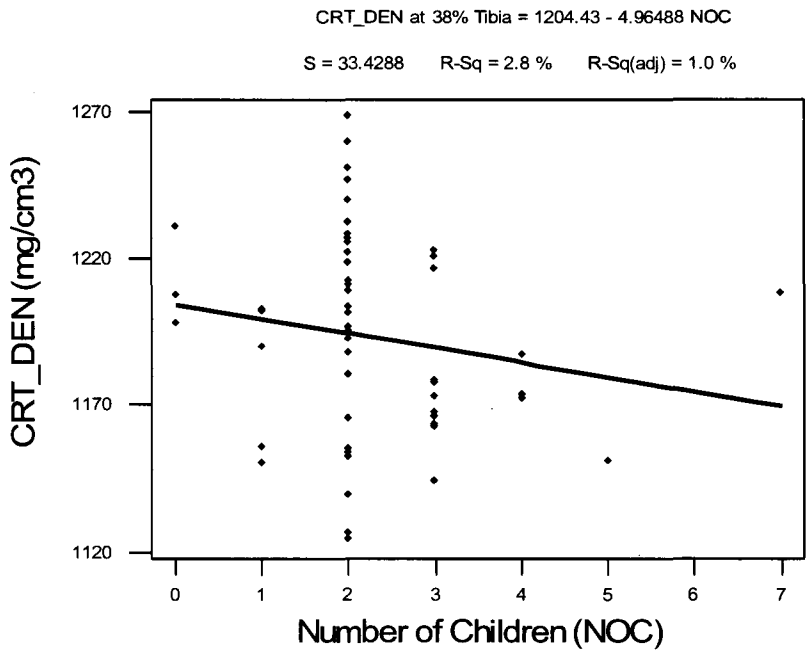
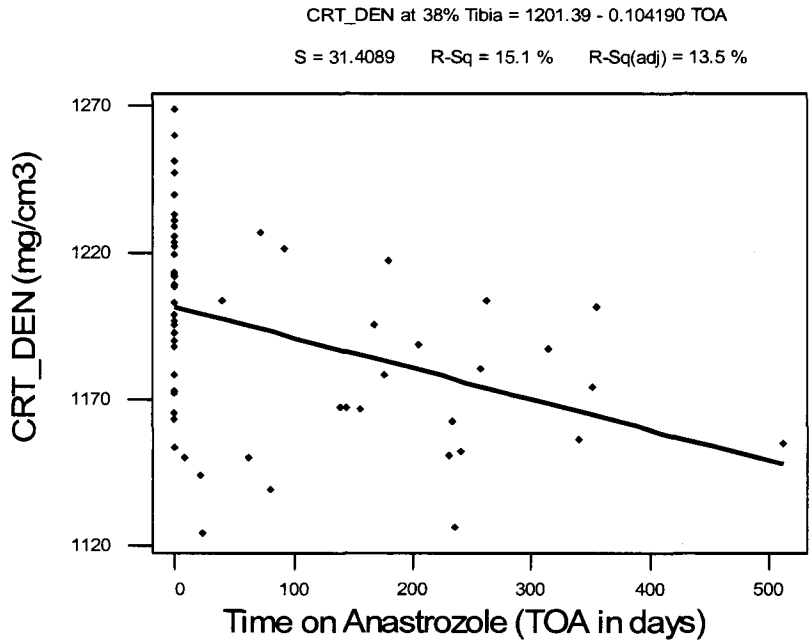


FIGURE 88: REGRESSION ANALYSIS OF CRT_DEN AND TOA AT THE 38% TIBIA



4.3.5 Correlation Analysis of Skeletal Sites and Anthropometric Characteristics

Correlation analyses were conducted to determine whether there was a significant association between the four measurement sites (4% radius and tibia, 20% radius, and 38% tibia). Also, an investigation into the relationship between trabecular, cortical, and total density values was undertaken. Negative correlation coefficients signify that the variables are related in an inverse manner, whereas, positive correlation coefficients indicate the variables are directly related. TOT_DEN and TRAB_DEN at the 4% radius significantly correlated with all parameters measured at the 4% tibia. TOT_CNT at the 4% radius was only significantly associated with TOT_CNT (r=0.74) and TRAB_CNT (r=0.62) at the 4% tibia; TRAB_CNT at the 4% radius was not associated with any of the 4% tibia parameters. At the 4% tibia, the only parameters significantly associated with the 20% radius were TOT_DEN (r=0.42) and TRAB_DEN (r=0.44); these correlated with 20% radius TOT_DEN.

When looking at the relationship between 4% and 20% radius measurements, TOT_CNT at the 4% site significantly correlated with all measurements at the 20% site. TOT_DEN at the 20% radius correlated with all but TRAB_CNT at the 4% site. At the 38% tibia, TOT_CNT and CRT_CNT correlated with TOT_DEN (r=0.49 and r=0.43 respectively) and TOT_CNT (r=0.64 and r=0.55 respectively) at the 4% radius, and with all parameters at the 20% radius. The 38% tibia TOT_CNT and CRT_CNT significantly correlated with the 4% tibia TOT_DEN (r=0.39) and TOT_CNT (r=0.58 and r=0.49 respectively). Each cell in Table 19 displays the Pearson Product Moment Correlation (r) between the intersecting variables and the associated p-values.

TABLE 19: PEARSON CORRELATION COEFFICIENTS AND P-VALUES BETWEEN SKELETAL SITES

Variable (n = 58)	Pearson Correlation Coefficients and P-Values (ns = not significant; *p< 0.05, ** p<0.01)											
	4% Radius TOT_DEN	4% Radius TOT_CNT	4% Radius TRAB_DEN	4% Radius TRAB_CNT	4% Tibia TOT_DEN	4% Tibia TOT_CNT	4% Tibia TRAB_DEN	4% Tibia TRAB_CNT	20% Radius TOT_DEN	20% Radius TOT_CNT	20% Radius CRT_DEN	20% Radius CRT_CNT
4% Tibia TOT_DEN	0.71 **	0.33 ns	0.60 **	0.09 ns	- -	- -	- -	- -	- -	- -	- -	- -
4% Tibia TOT_CNT	0.59 **	0.74 **	0.41 *	0.30 ns	- -	- -	- -	- -	- -	- -	- -	- -
4% Tibia TRAB_DEN	0.67 **	0.32 ns	0.58 **	0.12 ns	- -	- -	- -	- -	- -	- -	- -	- -
4% Tibia TRAB_CNT	0.53 **	0.62 **	0.40 *	0.28 ns	- -	- -	- -	- -	- -	- -	- -	- -
20% Radius TOT_DEN	0.69 **	0.53 **	0.63 **	0.26 ns	0.42 *	0.36 ns	0.44 *	0.36 ns	- -	- -	- -	- -
20% Radius TOT_CNT	0.20 ns	0.55 **	0.05 ns	0.18 ns	-0.15 ns	0.15 ns	-0.09 ns	0.15 ns	- -	- -	- -	- -
20% Radius CRT_DEN	0.62 **	0.48 *	0.32 ns	-0.00 ns	0.18 ns	0.27 ns	0.20 ns	0.24 ns	- -	- -	- -	- -
20% Radius CRT_CNT	0.33 ns	0.66 **	0.18 ns	0.25 ns	-0.04 ns	0.24 ns	0.01 ns	0.23 ns	- -	- -	- -	- -
38% Tibia TOT_DEN	0.19 ns	0.12 ns	-0.10 ns	-0.20 ns	0.12 ns	0.13 ns	0.01 ns	0.03 ns	0.08 ns	0.09 ns	0.21 ns	0.13 ns
38% Tibia TOT_CNT	0.49 *	0.64 **	0.28 ns	0.19 ns	0.39 *	0.58 **	0.24 ns	0.38 ns	0.55 **	0.44 *	0.55 **	0.59 **
38% Tibia CRT_DEN	0.11 ns	-0.11 ns	0.18 ns	-0.04 ns	0.02 ns	-0.26 ns	-0.05 ns	-0.27 ns	0.31 ns	0.24 ns	0.40 *	0.25 ns
38% Tibia CRT_CNT	0.43 *	0.55 **	0.16 ns	0.08 ns	0.33 ns	0.49 *	0.17 ns	0.28 ns	0.47 *	0.40 *	0.50 *	0.53 **

To further explore the relationship between each skeletal site measurement, correlation analyses were conducted to determine if there were any patterns of association between the four measurement sites (4% radius and tibia, 20% radius, and 38% tibia) and the anthropometric characteristics of the study subjects. In the course of this analysis, several interesting findings were revealed. Only the 20% radius was significantly negatively correlated with both age and years since menopause, and this was significant for TOT_DEN (r=-0.32 and r=-0.31 respectively), TOT_CNT (r=-0.37 and r=-0.31 respectively), CRT_DEN (r=-0.40 and r=-0.35 respectively), and CRT_CNT (r=-0.42 and r=-0.36 respectively). The only sites significantly associated with subjects' height were the 38% tibia TOT_CNT (r=0.35) and CRT_CNT (r=0.28). The 4% sites of the radius and tibia significantly positively correlated with mass and BMI. Only at the primarily cortical bone sites, the 20% radius and 38% tibia, TOT_CNT (r=-0.32 and r=-0.36 respectively) and CRT_CNT (r=-0.31 for both sites) measurements were significantly associated with the number of biological children in the sample population. With respect to time on Anastrozole (TOA), TOT_CNT at the 4% radius (r=-0.36), TOT_CNT (r=-0.33), CRT_CNT (r=-0.34) and CRT_DEN (r=-0.44) at the 20% radius and CRT_DEN (r=-0.39) and CRT_CNT (r=-0.27) at the 38% tibia were significantly negatively correlated with days on Anastrozole. When these data were adjusted for all the other variables, the significant association between TOA persisted for all sites except the 20% radius TOT_CNT (p=0.055). Each cell in Table 20 displays the Pearson Product Moment Correlation (r) between the intersecting variables and the associated p-values.

TABLE 20: PEARSON CORRELATION COEFFICIENTS AND P-VALUES BETWEEN SKELETAL SITES AND ANTHROPOMETRIC CHARACTERISTICS

Variable (n = 58)	Pearson Correlation Coefficients and P-Values (ns = not significant; *p< 0.05, ** p<0.01)															
	4% Radius TOT_DEN	4% Radius TOT_CNT	4% Radius TRAB_DEN	4% Radius TRAB_CNT	4% Tibia TOT_DEN	4% Tibia TOT_CNT	4% Tibia TRAB_DEN	4% Tibia TRAB_CNT	20% Radius TOT_DEN	20% Radius TOT_CNT	20% Radius CRT_DEN	20% Radius CRT_CNT	38% Tibia TOT_DEN	38% Tibia TOT_CNT	38% Tibia CRT_DEN	38% Tibia CRT_CNT
Age (years)	-0.18 ns	-0.21 ns	0.10 ns	0.13 ns	-0.03 ns	0.04 ns	0.08 ns	0.13 ns	-0.32 *	-0.37 **	-0.40 **	-0.42 **	0.08 ns	-0.27 *	-0.22 ns	-0.24 ns
Height (m)	-0.05 ns	0.11 ns	-0.13 ns	-0.03 ns	-0.06 ns	0.15 ns	-0.00 ns	0.17 ns	0.00 ns	0.13 ns	0.15 ns	0.15 ns	-0.25 ns	0.35 **	-0.05 ns	0.28 *
Mass (kg)	0.31 *	0.38 **	0.21 ns	0.17 ns	0.38 **	0.48 **	0.28 *	0.33 *	0.13 ns	0.07 ns	0.05 ns	0.10 ns	0.06 ns	0.26 *	0.02 ns	0.24 ns
Body Mass Index (kg/m2)	0.31 *	0.32 *	0.26 *	0.17 ns	0.41 **	0.40 **	0.29 *	0.25 *	0.11 ns	0.00 ns	-0.02 ns	0.02 ns	0.16 ns	0.10 ns	0.05 ns	0.10 ns
Years Since Menopause (years)	-0.13 ns	-0.17 ns	0.04 ns	0.04 ns	-0.07 ns	0.11 ns	0.12 ns	0.25 ns	-0.31 *	-0.31 *	-0.35 **	-0.36 *	0.00 ns	-0.23 ns	-0.23 ns	-0.22 ns
Age at Menopause (years)	0.01 ns	0.02 ns	0.07 ns	0.08 ns	0.10 ns	-0.03 ns	-0.01 ns	-0.10 ns	0.10 ns	0.06 ns	0.05 ns	0.05 ns	0.09 ns	-0.01 ns	0.06 ns	-0.02 ns
Number of Children	-0.04 ns	-0.21 ns	-0.04 ns	-0.14 ns	-0.18 ns	0.02 ns	-0.06 ns	-0.10 ns	-0.11 ns	-0.32 *	-0.22 ns	-0.31 *	0.07 ns	-0.36 **	-0.17 ns	-0.31 *
Time on Anastrozole (days)	-0.23 ns	-0.36 **	-0.15 ns	-0.19 ns	-0.21 ns	-0.19 ns	-0.09 ns	-0.07 ns	-0.17 ns	-0.33 *	-0.44 **	-0.34 *	-0.07 ns	-0.24 ns	-0.39 **	-0.27 *

4.3.6 Muscle, Subcutaneous Fat, and Bone Cross-Sectional Area

pQCT scans were performed on the non-dominant tibia at the 66% site. As noted previously, positioning at the 66% tibia site was occasionally difficult due to the size of the leg aperture in relation to the diameter of a subject's calf. Table 21 lists the results for muscle, fat, and bone area in healthy postmenopausal subjects (n=31) and breast cancer subjects (n=27) assessed at the 66% tibia site. There were no statistically significant differences between the two subject populations for these parameters at this site. Overall, the mean values for muscle, fat, and bone area were somewhat lower in the breast cancer subjects.

TABLE 21: CROSS-SECTIONAL MUSCLE, SUBCUTANEOUS FAT AND BONE AREA AT THE 66% TIBIA

Variable[a]	All Subjects (n = 58)	Healthy Post-Menopausal Subjects (n = 31)	Breast Cancer Subjects (n = 27)
Area (mm^2)			
Total muscle	5588 (76)	5669(104)	5496 (110)
Total fat	4907 (184)	4951 (305)	4868 (224)
Total bone[b]	327 (5)	331 (6)	323 (9)

[a]Data expressed as the mean (SEM); [b]includes total tibia and fibula

Subsequently, correlation analysis was conducted to reveal any associations between the four skeletal measurement sites (4% radius and tibia, 20% radius, and 38% tibia) and the muscle, fat, and bone area data assessed at the 66% tibia site (Table 22). Total bone area at the 66% tibia significantly correlated with most of the pQCT parameters at all four measurement sites. Total fat area only significantly correlated with the TOT_CNT (r=0.29) and TOT_DEN (r=0.30) at the 4% tibia. Total muscle area significantly correlated with distal and diaphyseal tibia TOT_CNT (r=0.39 and r=0.28 respectively), diaphyseal tibia CRT_CNT (r=0.30), and distal radius TOT_CNT (r=0.30).

TABLE 22: CORRELATION BETWEEN SKELETAL SITES AND MUSCLE, SUBCUTANEOUS FAT AND BONE AREA AT THE 66% TIBIA

Variable (n = 58)	Pearson Correlation Coefficients and P-Values (ns = not significant; *p< 0.05, ** p<0.01)															
	4% Radius TOT_DEN	4% Radius TOT_CNT	4% Radius TRAB_DEN	4% Radius TRAB_CNT	4% Tibia TOT_DEN	4% Tibia TOT_CNT	4% Tibia TRAB_DEN	4% Tibia TRAB_CNT	20% Radius TOT_DEN	20% Radius TOT_CNT	20% Radius CRT_DEN	20% Radius CRT_CNT	38% Tibia TOT_DEN	38% Tibia TOT_CNT	38% Tibia CRT_DEN	38% Tibia CRT_CNT
Total Muscle Area at 66% Tibia (mm^2)	0.08 ns	0.30 *	0.04 ns	0.18 ns	0.10 ns	0.39 **	0.03 ns	0.24 ns	0.01 ns	0.17 ns	0.04 ns	0.16 ns	0.21 ns	0.28 *	-0.11 ns	0.30 *
Total Fat Area at 66% Tibia (mm^2)	0.22 ns	0.25 ns	0.09 ns	0.06 ns	0.30 *	0.29 *	0.17 ns	0.14 ns	0.07 ns	-0.11 ns	-0.08 ns	-0.12 ns	-0.08 ns	0.16 ns	-0.06 ns	0.10 ns
Total Bone Area at 66% Tibia (mm^2)	0.42 **	0.54 **	0.31 *	0.23 ns	0.45 **	0.67 **	0.49 **	0.61 **	0.37 **	0.49 **	0.36 **	0.54 **	0.18 ns	0.77 **	0.16 ns	0.77 **

4.4 Correlation Analysis of pQCT Parameters, DXA Measurements, and Serum Parathyroid Hormone, Vitamin D, Calcium, and Phosphate Levels in Women with a History of Breast Carcinoma

A further objective of this study was to investigate the relationship between DXA measurements of BMD and BMC, and the pQCT measurement parameters which were the focus of this research. The sub-group of breast cancer subjects (n=27) underwent DXA scans of the lumbar spine, hip, and total body as well as serum analysis. The densitometric data were subdivided by measurement sites such that data sets for the hip, lumbar spine, forearm, leg and whole body were each analyzed separately. The serum levels of vitamin D, parathyroid hormone (PTH), calcium, and phosphate were abstracted from patient charts and analyzed.

Pearson Product Moment Correlation (r) and the associated p-values were calculated to determine if significant relationships existed between these measurements and the pQCT variables assessed at the radius and tibia in these same study subjects. Interestingly, the 4% distal tibia TOT_CNT significantly correlated with all the DXA measurement sites. Furthermore, the TOT_CNT at the 4% radius significantly ($p<0.01$) correlated with all the BMC measurements assessed via DXA; this included the lumbar spine BMC, total hip BMC, non-dominant arm and leg BMC, and total body BMC. The 4% tibia TRAB_CNT significantly associated with the total hip BMC ($p<0.05$), the non-dominant arm and leg BMC ($p<0.01$) and the total body BMC ($p<0.01$). The 20% radius TOT_CNT was only significantly associated with the lumbar spine BMC and the arm BMC, however the 20% radius CRT_CNT was significantly associated with every BMC variable as was the 38% tibia TOT_CNT and 38% tibia CRT_CNT. The 38% tibia TOT_CNT and CRT_CNT also

significantly associated with the DXA-derived measurements of lean muscle mass at both the non-dominant arm and leg.

With respect to the blood serum levels, the mean values for each parameter were well within the normal recommended range. The mean (SD) level for vitamin D was 79.5 (30.3) (normal range of 22.5-94.0 nmol/L), 4.5 (3.9) for PTH (normal range of 1.6-6.9 pmol/L), 2.4 (0.14) for calcium (normal range of 2.15-2.55 nmol/L), and 1.2 (0.14) for blood phosphorus (normal range of 0.80-1.45 nmol/L). The only measurement that was significantly associated with the serum vitamin D level was the CRT_DEN (r=0.54, p=0.007) at the 38% tibia. PTH was significantly associated with the TRAB_DEN (r=-0.42, p=0.04) at the 4% radius. Descriptive results of the lumbar spine, total hip, arm, leg and total body DXA data are displayed in Table 23.

TABLE 23: SERUM LEVELS AND DXA MEASUREMENT VALUES IN BREAST CANCER SUBJECTS

Variable (n = 27)	Mean (SD)	Pearson Correlation P-Values (ns = not significant; *p< 0.05, ** p<0.01)															
		4% Radius TOT_DEN	4% Radius TOT_CNT	4% Radius TRAB_DEN	4% Radius TRAB_CNT	4% Tibia TOT_DEN	4% Tibia TOT_CNT	4% Tibia TRAB_DEN	4% Tibia TRAB_CNT	20% Radius TOT_DEN	20% Radius TOT_CNT	20% Radius CRT_DEN	20% Radius CRT_CNT	38% Tibia TOT_DEN	38% Tibia TOT_CNT	38% Tibia CRT_DEN	38% Tibia CRT_CNT
Lumbar Spine BMC	57.0 (9.1)	ns	**	ns	ns	ns	*	ns	ns	ns	**	ns	**	ns	**	ns	**
Lumbar Spine BMD	0.95 (0.1)	ns	ns	*	ns	ns	*	ns	ns	ns	ns	*	*	ns	*	ns	ns
Total Hip BMC	30.9 (5.0)	*	**	ns	ns	**	**	*	*	ns	ns	ns	ns	ns	**	ns	**
Femoral Neck BMD	0.72 (0.1)	ns	ns	ns	ns	**	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Non-dominant Arm[a]																	
BMC	140 (21)	ns	**	ns	*	*	**	ns	**	ns	*	ns	*	ns	*	ns	*
Fat	2586 (1257)	ns	ns	ns	*	*	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Lean Muscle	2021 (422)	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Total Mass	4747 (1642)	ns	ns	ns	ns	*	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
% Fat	52 (8)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Non-dominant Leg																	
BMC	366 (55)	ns	**	ns	ns	*	**	*	**	ns	ns	ns	*	ns	**	ns	**
Fat	4392 (1267)	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Lean Muscle	6175 (1142)	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	*	*	**
Total Mass	10933 (2311)	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
% Fat	40 (4)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Total Body																	
BMC	2056 (277)	ns	**	ns	ns	ns	**	ns	**	ns	ns	*	*	ns	**	ns	**
Fat	28545 (9809)	ns	ns	ns	ns	ns	**	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Lean Muscle	41602 (5835)	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	*	*	*
Total Mass	72204 (15056)	ns	*	ns	ns	*	**	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
% Fat	39 (6)	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Serum Levels[a]																	
Vitamin D	79.5 (30.3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns
PTH	4.5 (3.9)	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Calcium	2.4 (0.14)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Phosphate	1.2 (0.14)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]Data set consists of n=26 breast cancer subjects

4.5 pQCT Measurements of Trabecular and Cortical Bone After 12 and 24 Months of Anastrozole Therapy

The final objective of this thesis was to assess the effects of Anastrozole therapy on the pQCT assessed bone and muscle variables. Using the same pQCT protocol as described previously in section *3.3*, follow-up pQCT scans were performed approximately 12 and 24 months after the date of the baseline scans. Three individuals originally recruited to participate were unable to return for re-scanning at the 24 month time point, therefore, their 2-year data is not included in the study. At baseline the mean (SD) age of the study participants was 59 (6), after 1.0 ± 0.1 yr from the date of their baseline pQCT scans the mean age was 60 (6) and after 1.9 ± 0.1 yr from the date of the baseline scan the mean age was 58 (6). Descriptive anthropometric data for the study subjects are listed in Table 24.

TABLE 24: ANTHROPOMETRIC DATA OF THE STUDY PARTICIPANTS

Variable[a]	Baseline ($n = 8$)	12 Month Follow-up ($n = 8$)	24 Month Follow-up ($n = 5$)
Age (years)	59 (6)	60 (6)	58 (6)
Age at Menopause (years)	45 (6)	45 (6)	44 (2)
Years Since Menopause (years)	13 (9)	14 (9)	13 (8)
Height (m)	1.64 (0.05)	1.64 (0.05)	1.63 (0.05)
Mass (kg)	85.6 (12.3)	85.6 (12.3)	89.4 (14.0)
Body Mass Index (kg/m^2)	31.9 (4.3)	31.9 (4.3)	33.7 (4.6)
Number of Biological Children	2 (0.5)	2 (0.5)	2 (0.4)

[a]Data expressed as the mean (SD)

Baseline and repeated measurements of bone density, mass, and geometry data are presented as dot-plots in Figures 89 to 92. Participants who were prescribed medications for osteopenia or osteoporosis during the course of the study were examined independently. Of the 8 participants, one began taking a bisphosphonate shortly after the baseline scan was

completed. The changes in bone and muscle outcome variables at the radius and tibia in this cohort of breast cancer subjects were assessed using linear mixed effects ANOVA. Variables were analyzed in groups according to measurement time; baseline, 1 year, and 2 year measurements. The pQCT data were further subdivided by measurement site, such that the distal and diaphyseal sites of the radius and tibia were assessed separately. The pQCT muscle and subcutaneous fat data was also analyzed independently of the bone data.

At the distal radius, the mean values for TOT_CNT, TRAB_CNT, TOT_DEN and TRAB_DEN appeared to remain relatively constant over the 2 year period; there was no statistically significant change in the mean for any of these variables ($p>0.05$). The results were similar at the 4% distal tibia, with no statistically significant difference in the mean values for total or trabecular bone density and content. However, at the 20% diaphyseal radius, there was a significant decrease in CRT_DEN ($p=0.025$) during the 2 year follow-up. Also at the 38% diaphyseal tibia, the decrease in CRT_DEN closely approached significance at $p=0.051$. The ANOVA analysis as well as box plots of the data for CRT_DEN at the 20% radius and 38% tibia are displayed in Figures 93 and 94 respectively. At the 66% tibia site, any changes in total bone, muscle, and fat area over the two year period were assessed. The trend was towards a slight increase in the mean values for fat and muscle area and a slight decrease in bone area over the two year period; however, utilizing ANOVA these changes were not statistically significant. Baseline and repeated measurements of muscle, fat, and bone area are presented as dot-plots in Figure 95.

FIGURE 89: BASELINE, 1 YEAR, AND 2 YEAR FOLLOW-UP PLOTS - 4% RADIUS

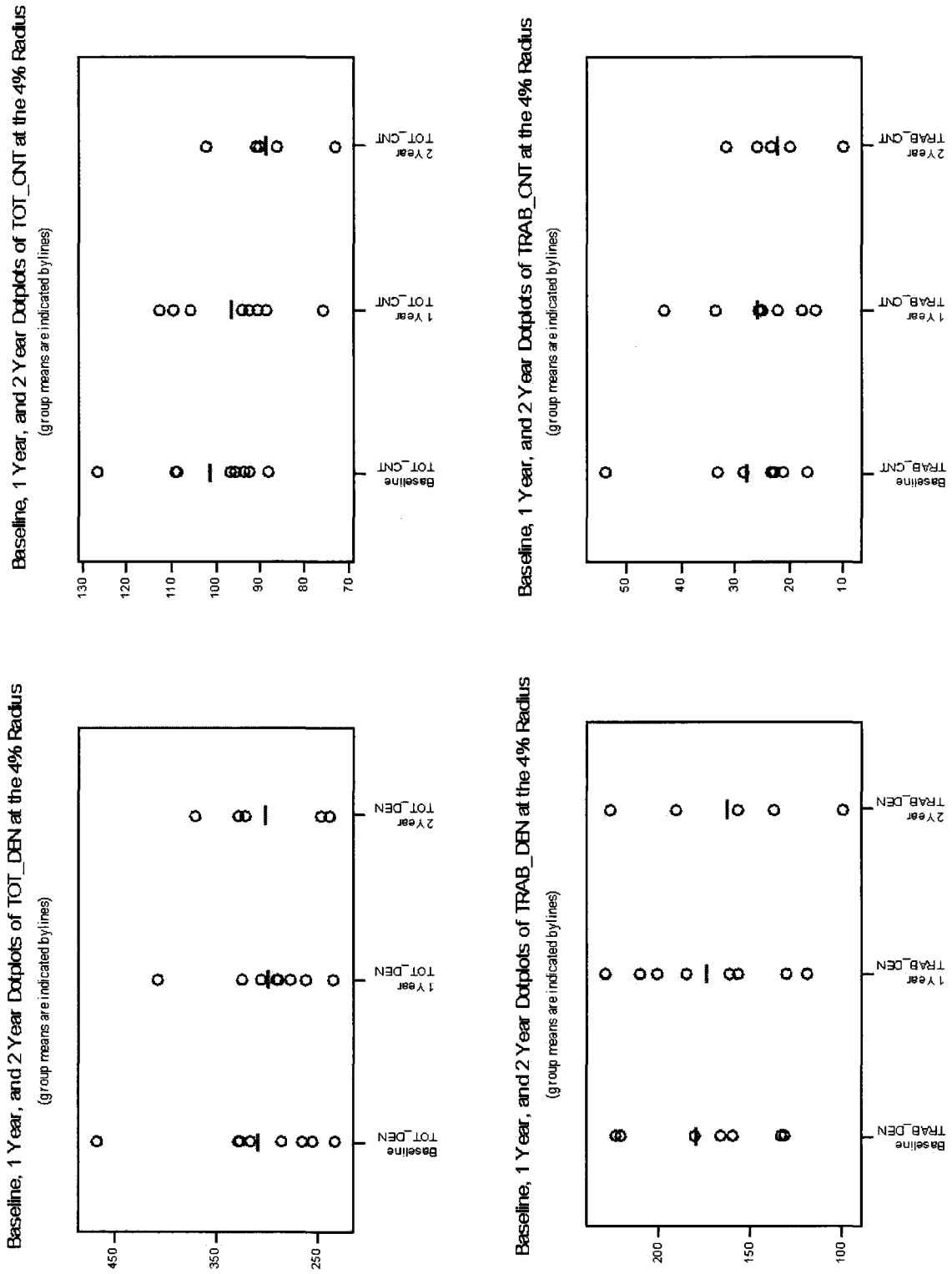


FIGURE 90: BASELINE, 1 YEAR, AND 2 YEAR FOLLOW-UP PLOTS - 4% TIBIA

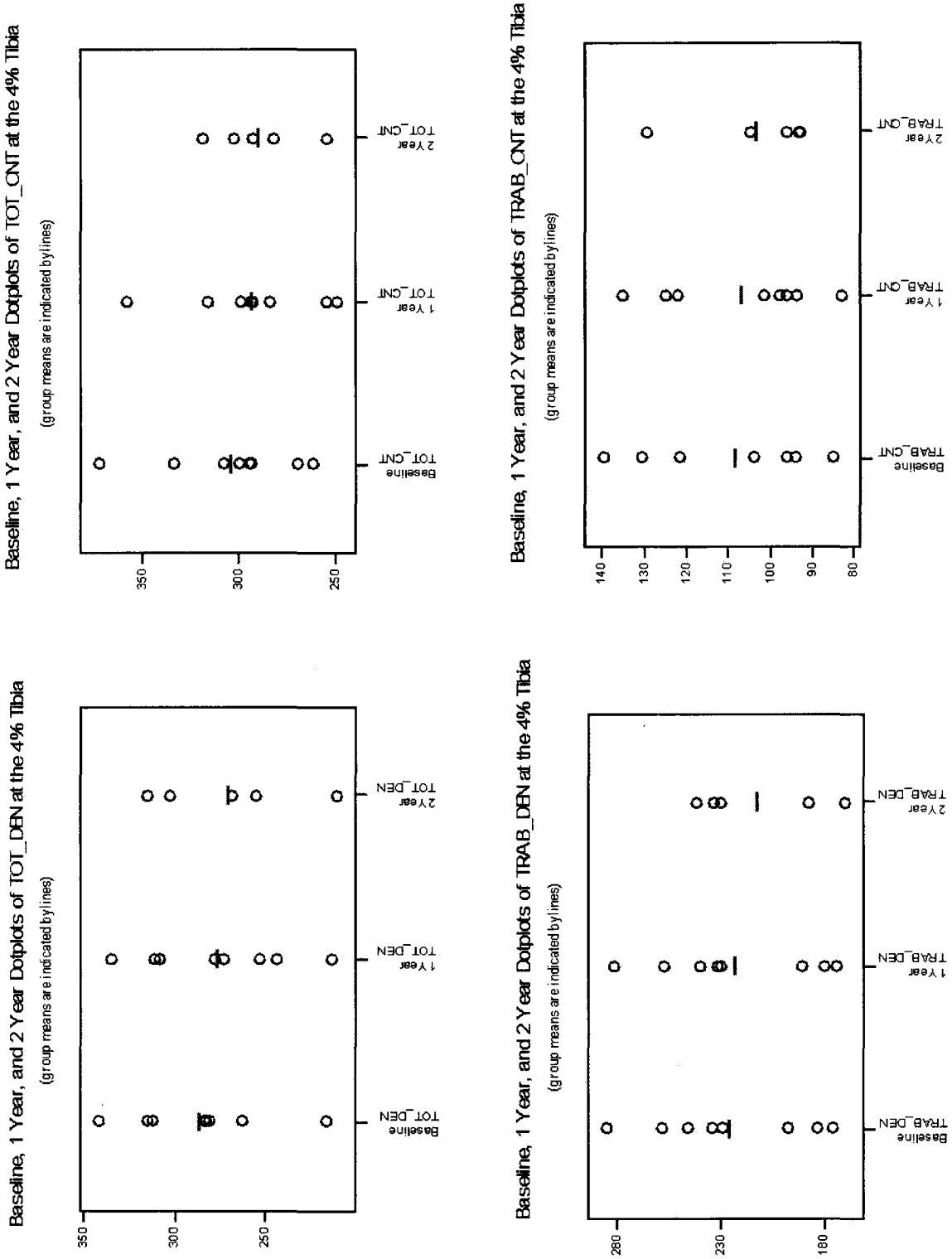


FIGURE 91: BASELINE, 1 YEAR, AND 2 YEAR FOLLOW-UP PLOTS - 20% RADIUS

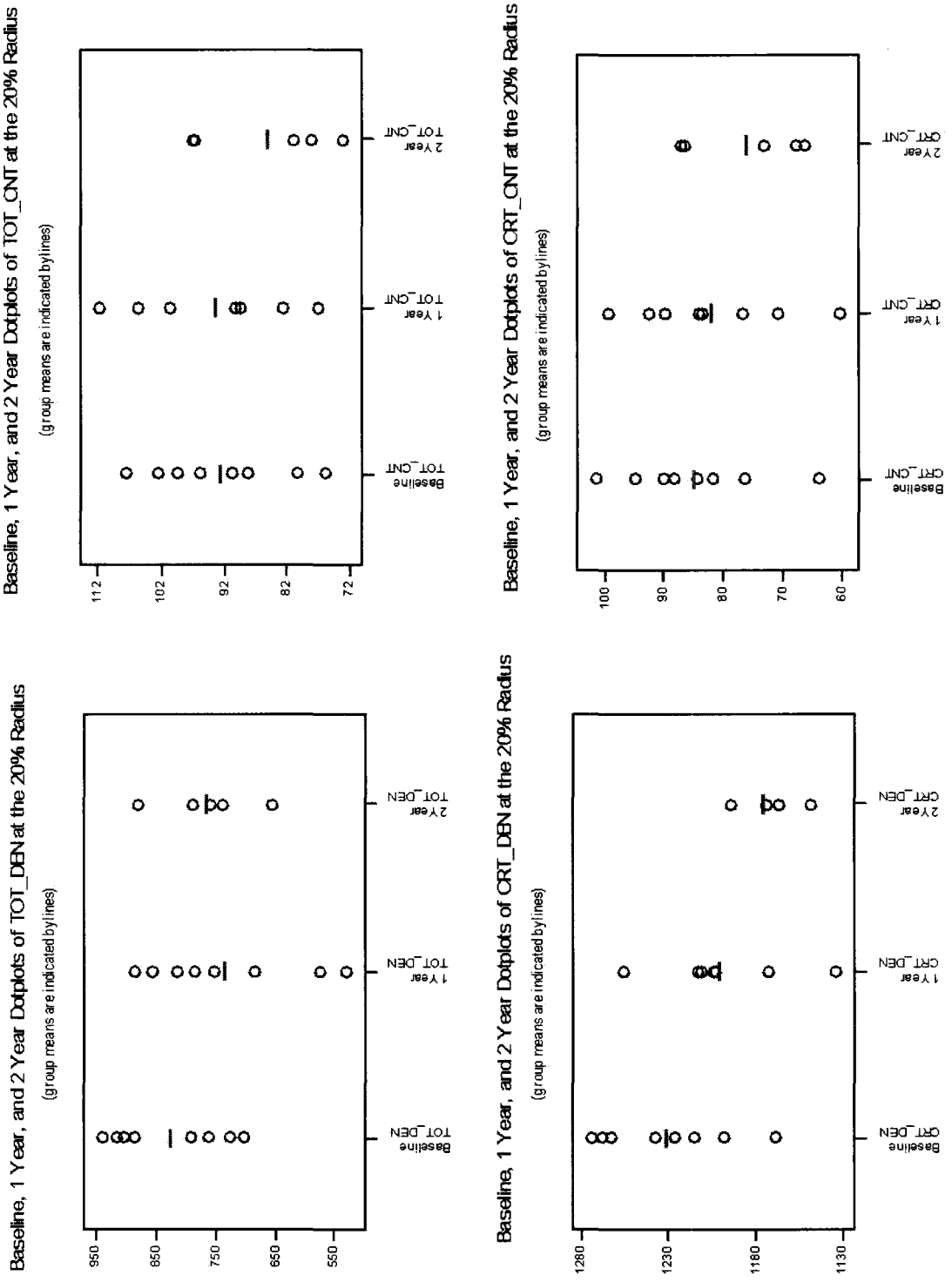


FIGURE 92: BASELINE, 1 YEAR, AND 2 YEAR FOLLOW-UP PLOTS - 38% TIBIA

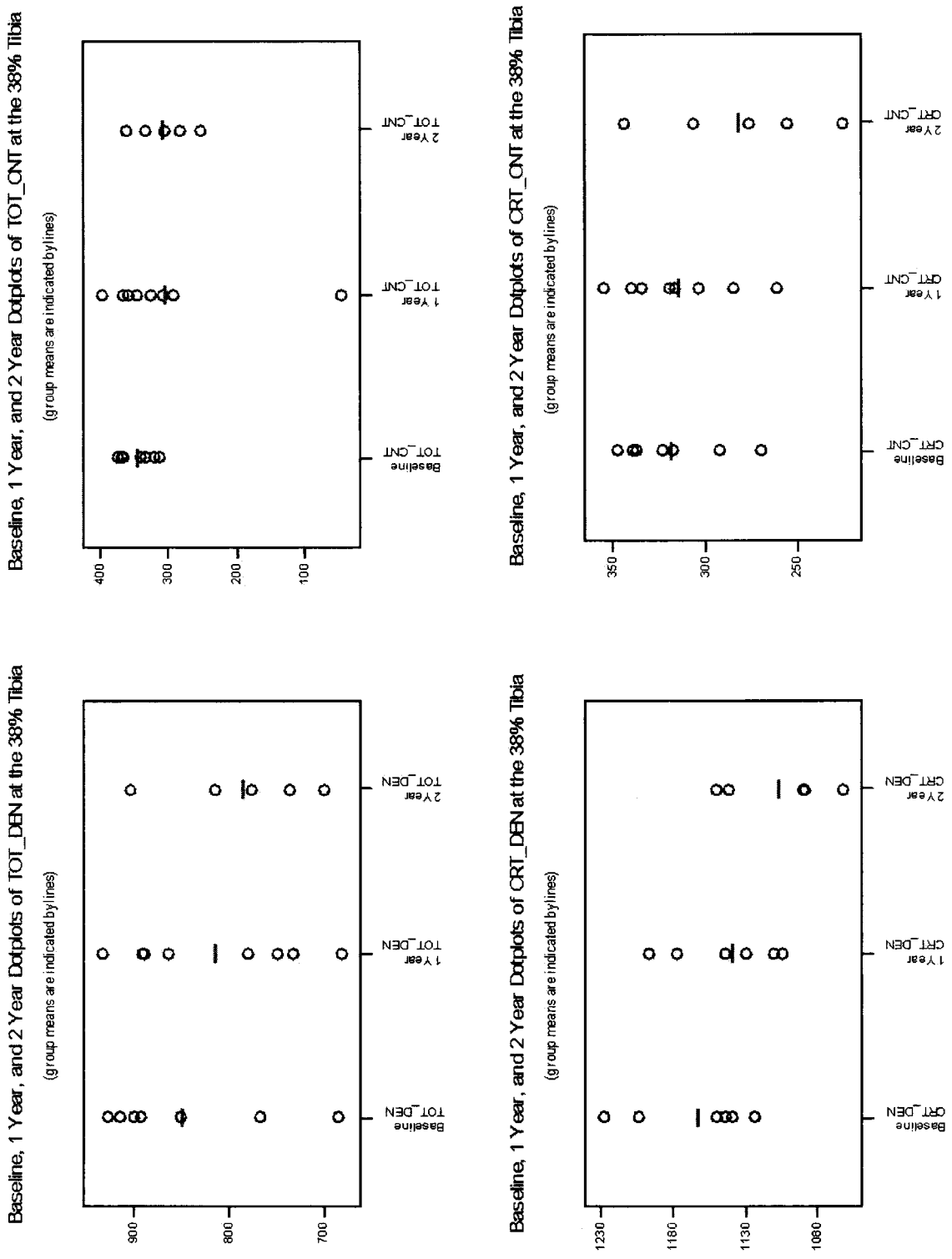


FIGURE 93: BASELINE, 1 YEAR, AND 2 YEAR ANOVA AND BOXPLOTS OF CRT_DEN AT THE 20% RADIUS (*ANOVA P=0.025*)

	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
BCRT_DEN	8	1231.1	36.9	(------*------)
1CRT_DEN	8	1201.0	35.0	(------*------)
2CRT_DEN	5	1175.4	19.3	(--------*--------)
Pooled StDev =		33.0		1155 1190 1225 1260

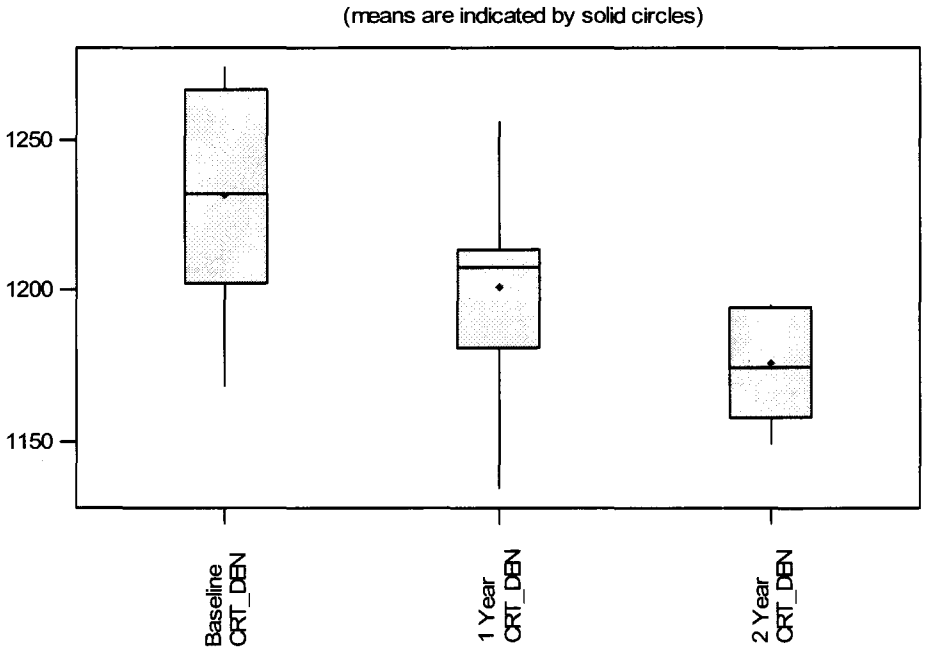


FIGURE 94: BASELINE, 1 YEAR, AND 2 YEAR ANOVA AND BOXPLOTS OF CRT_DEN AT THE 38% TIBIA (*ANOVA P=0.051*)

```
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
            N      Mean     StDev ----+---------+---------+---------+--
BCRT_DEN    8    1162.6      37.7                    (-------*-------)
1CRT_DEN    8    1139.4      33.0               (-------*------)
2CRT_DEN    5    1106.7      37.3     (---------*---------)
                                  ----+---------+---------+---------+--
Pooled StDev =     35.7             1085      1120      1155      1190
```

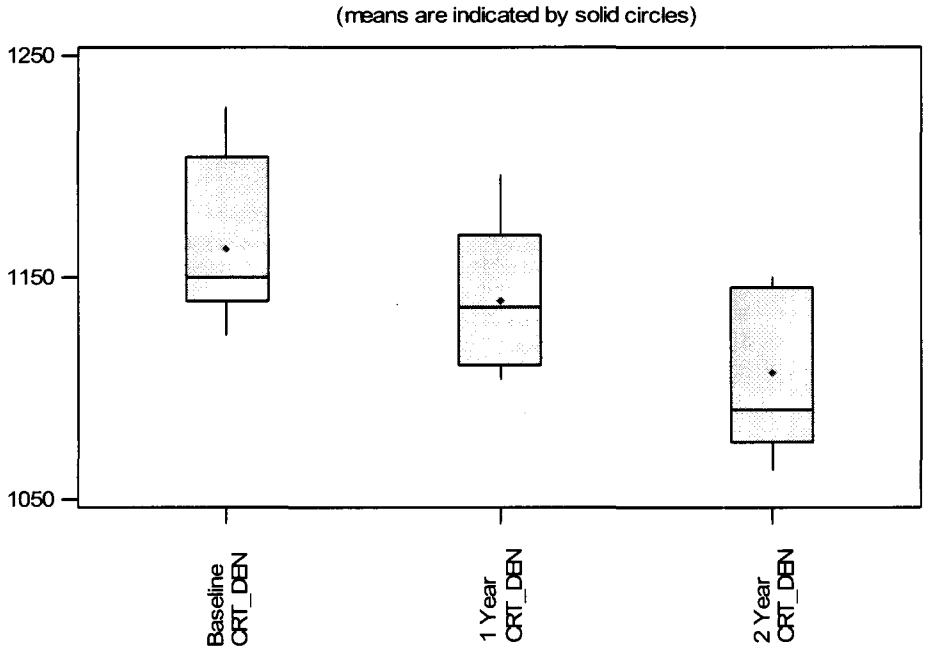
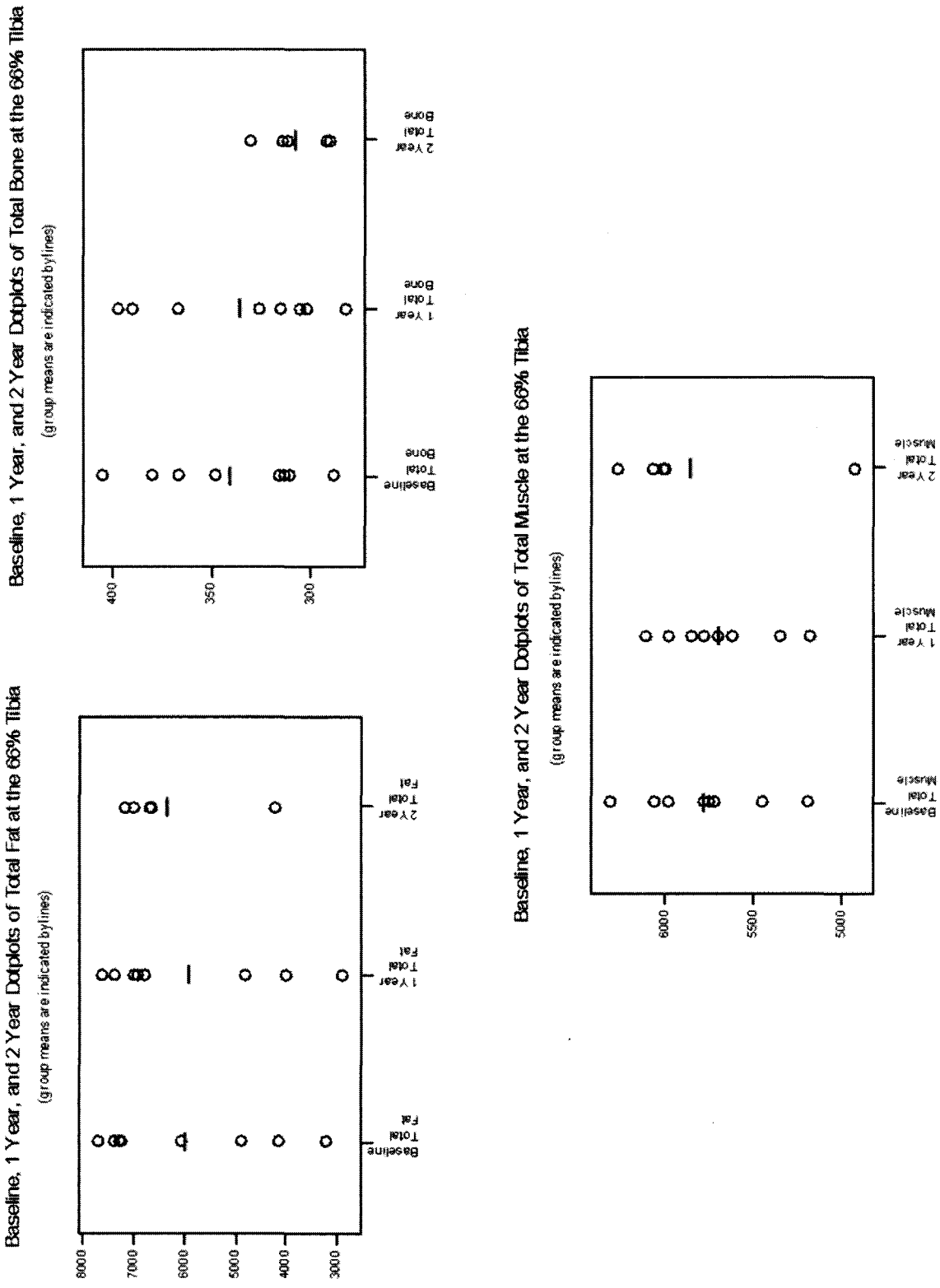
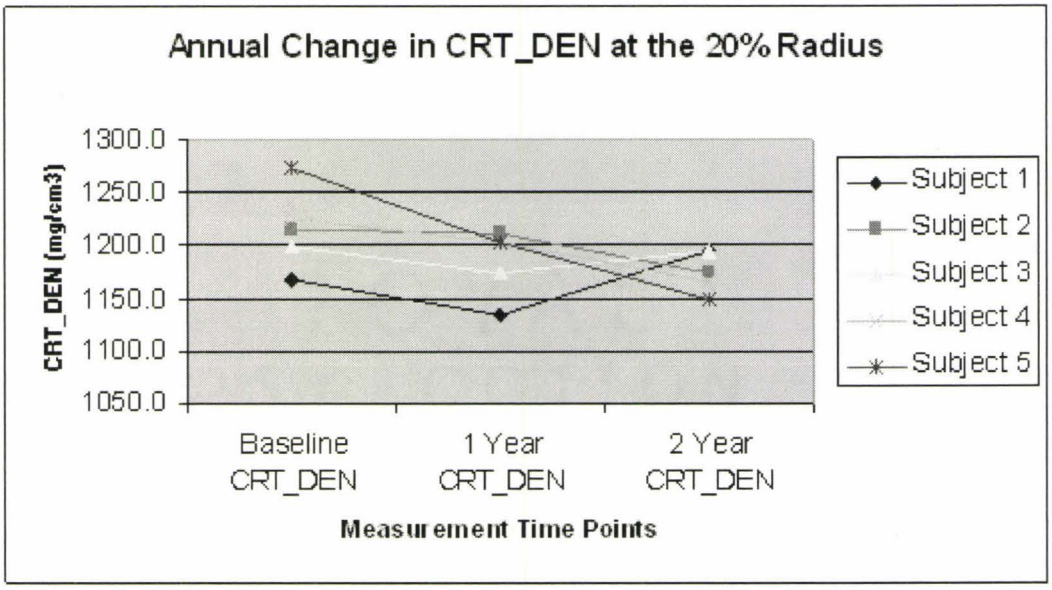


FIGURE 95: BASELINE, 1 YEAR, AND 2 YEAR FOLLOW-UP PLOTS - 66% TIBIA



Subsequently, the five study subjects that had all three follow-up measurements conducted were analyzed to determine the percentage change in CRT_DEN between baseline and 12 months of treatment and from 12 to 24 months of treatment. The annual change in CRT_DEN is displayed in Figure 96. At the 20% radius, from baseline to 12 months of Anastrozole, all subjects experienced a decrease in CRT_DEN that ranged from -0.3% to -5.5%. During the second years of treatment, three subjects experienced a further decrease (from -3.1% to -4.5%) whereas two subjects experienced an increase of 1.8% and 5.2%. When this data was further analyzed by linear regression to acquire the percentage change per year, one subject experienced a positive increase in CRT_DEN of 1.1%, and the other four experienced a decrease. It is interesting to note that the only subject that experienced the increase, Subject 1, was the participant that had started taking a bisphosphonate shortly after starting Anastrozole. At the 38% tibia, from baseline to 12 months of Anastrozole treatment, all subjects experienced a decrease in CRT_DEN that ranged from -0.6% to -3.4%. During the second years of treatment, four subjects experienced a further decrease (from -0.1% to -4.3%) whereas one subject experienced an increase of 0.6%. When these data were further analyzed by linear regression, the same observation made at the radius was also reflected at the tibia. Subject 1, whom was taking a bisphosphonate, experienced the smallest decrease of CRT_DEN at -0.4%. Tables 25 and 26 display the percentage change at the 20% radius and Tables 27 and 28 display the percentage change at the 38% tibia. A significant positive association (R^2=94.9%) between percentage change in CRT_DEN at the radius and tibia (r=0.97, p=0.005) was determined (Figure 97).

FIGURE 96: ANNUAL CHANGE IN CRT_DEN AT THE 20% RADIUS AND 38% TIBIA



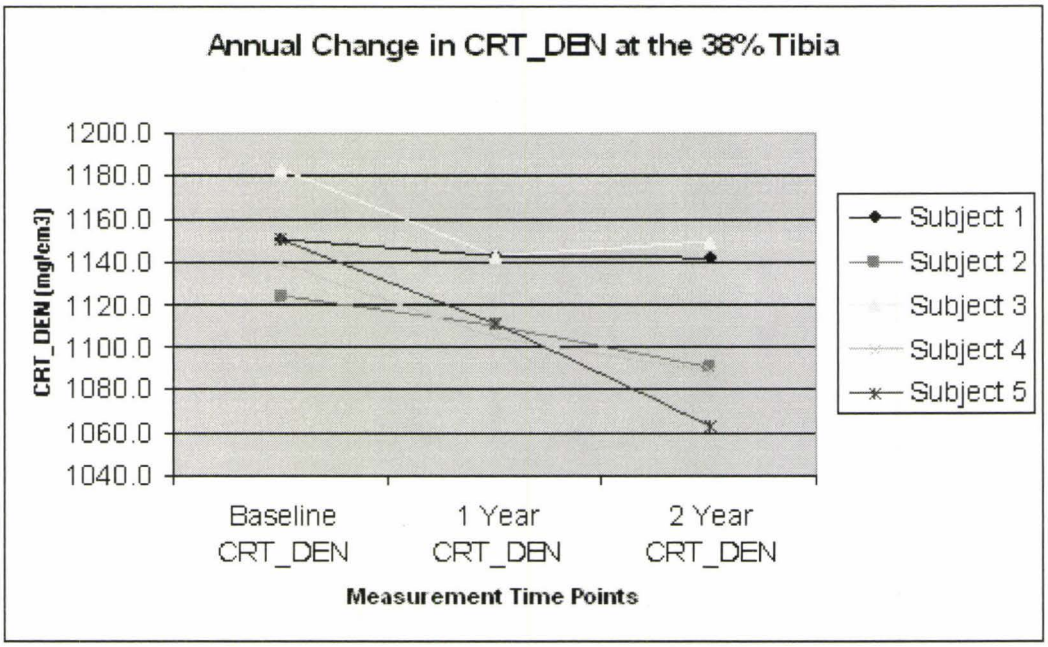


TABLE 25: CRT_DEN AT THE 20% RADIUS AFTER 12 AND 24 MONTHS OF ANASTROZOLE

Subject	20% Radius CRT_DEN Baseline *(mg/cm^3)*	20% Radius CRT_DEN 12 Months *(mg/cm^3)*	Change From Baseline to 12 Months	Percentage Change From Baseline to 12 Months	20% Radius CRT_DEN 24 Months *(mg/cm^3)*	Change From 12 to 24 Months	Percentage Change From 12 to 24 Months
1	1168.3	1134.6	-33.7	-2.9	1193.6	59.0	5.2
2	1215.2	1211.1	-4.1	-0.3	1173.9	-37.2	-3.1
3	1197.8	1173.2	-24.6	-2.1	1194.5	21.3	1.8
4	1225.9	1203.2	-22.7	-1.9	1166.3	-36.9	-3.1
5	1273.6	1203.6	-70.0	-5.5	1148.9	-54.7	-4.5

TABLE 26: CRT_DEN AT THE 20% RADIUS – REGRESSION USING ALL 3 DATA POINTS

Subject	Y-intercept *(mgcm^{-3})*	Slope *(mgcm^{-3}y^{-1})*	Percentage Change per Year (%)
1	1152.85	-12.65	1.1
2	1220.72	-20.65	-1.7
3	1190.15	-1.65	-0.1
4	1228.27	-29.8	-2.4
5	1271.05	-62.35	-4.9

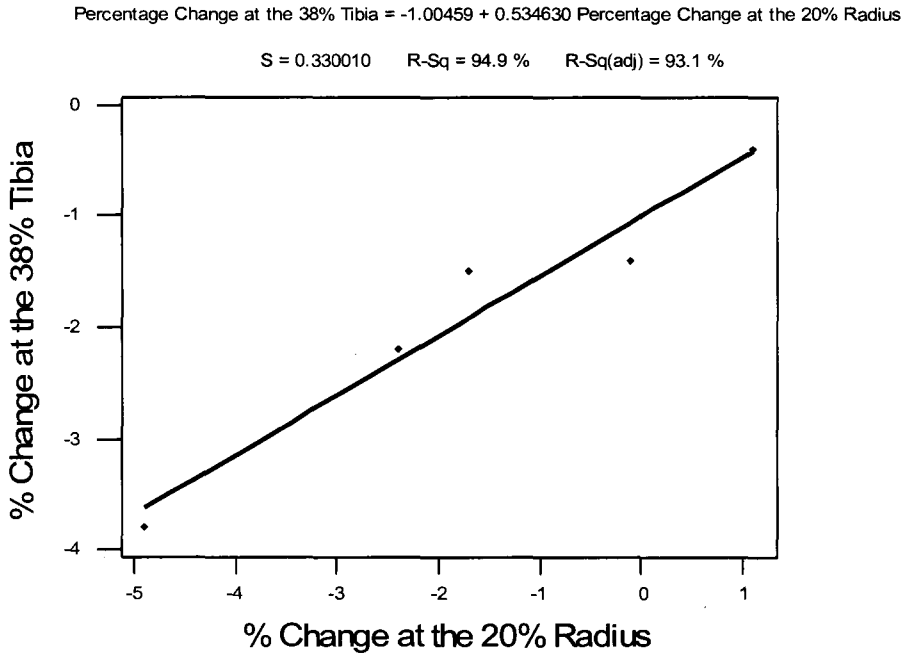
TABLE 27: CRT_DEN AT THE 38% TIBIA AFTER 12 AND 24 MONTHS OF ANASTROZOLE

Subject	38% Tibia CRT_DEN Baseline *(mg/cm^3)*	38% Tibia CRT_DEN 12 Months *(mg/cm^3)*	Change From Baseline to 12 Months	Percentage Change From Baseline to 12 Months	38% Tibia CRT_DEN 24 Months *(mg/cm^3)*	Change From 12 to 24 Months	Percentage Change From 12 to 24 Months
1	1150.3	1143.3	-7.0	-0.6	1141.6	-1.7	-0.1
2	1124.1	1110.4	-13.7	-1.2	1090.8	-19.6	-1.8
3	1182.7	1143.3	-39.4	-3.3	1149.7	6.4	0.6
4	1139.0	1104.5	-34.5	-3.0	1088.5	-16.0	-1.4
5	1150.1	1110.9	-39.2	-3.4	1063.1	-47.8	-4.3

TABLE 28: CRT_DEN AT THE 38% TIBIA – REGRESSION USING ALL 3 DATA POINTS

Subject	Y-intercept *(mgcm^{-3})*	Slope *(mgcm^{-3}y^{-1})*	Percentage Change per Year (%)
1	1149.42	-4.35	-0.4
2	1125.08	-16.65	-1.5
3	1175.07	-16.5	-1.4
4	1135.90	-25.25	-2.2
5	1151.53	-43.5	-3.8

FIGURE 97: REGRESSION ANALYSIS OF PERCENTAGE CHANGE IN CRT_DEN AFTER TWO YEARS OF ANASTROZOLE THERAPY



4.6 Cortical Density at Various Time Points of Anastrozole Treatment

Sixteen breast cancer subjects undergoing Anastrozole therapy were measured via pQCT at two time points during treatment. The objective was to determine if a trend in bone change could be detected. Nine subjects experienced a decrease in cortical bone density at the 20% radius, and seven subjects experienced an increase. At the 38% tibia, six experienced a decrease in cortical bone density and ten experienced an increase. A significant positive association (R^2=37.7%) between percentage change in CRT_DEN at the radius and tibia (r=0.61, p=0.01) was determined. Figure 98 displays the regression analysis, including the regression equation and R^2 values and Table 29 displays the measurement time points (days on Anastrozole), the CRT_DEN values at each site, and the percentage change in CRT_DEN between the two time points for each study subject.

FIGURE 98: REGRESSION ANALYSIS OF PERCENTAGE CHANGE IN CRT_DEN

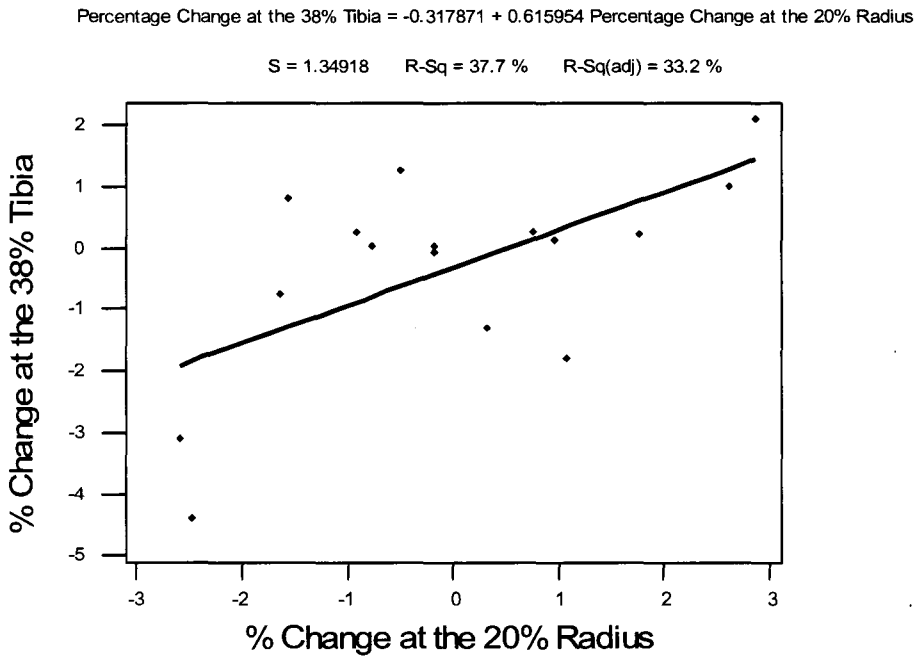


TABLE 29: CRT_DEN AT THE 20% RADIUS AND 38% TIBIA AFTER 12 MONTHS OF ANASTROZOLE AT VARIOUS TIME POINTS OF TREATMENT

Subject	pQCT Measurement Time Point 'A' (days on Anastrozole)	pQCT Measurement Time Point 'B' (days on Anastrozole)	20% Radius CRT_DEN at Time 'A'	20% Radius CRT_DEN at Time 'B'	20% Radius CRT_DEN Change From Time 'A' to Time 'B'	20% Radius CRT_DEN % Change From Time 'A' to Time 'B'	38% Tibia CRT_DEN at Time 'A'	38% Tibia CRT_DEN at Time 'B'	38% Tibia CRT_DEN Change From Time 'A' to Time 'B'	38% Tibia CRT_DEN % Change From Time 'A' to Time 'B'
1	139	504	1176.9	1167.9	-9.0	-0.76	1167.2	1167.6	0.4	0.03
2	157	522	1245.3	1233.9	-11.4	-0.92	1166.3	1169.3	3.0	0.26
3	169	534	1277.6	1246.0	-31.6	-2.47	1195.4	1142.7	-52.7	-4.41
4	180	545	1200.6	1221.9	21.3	1.77	1216.9	1219.7	2.8	0.23
5	206	571	1225.3	1260.3	35.0	2.86	1188.1	1213.3	25.2	2.12
6	230	595	1222.6	1231.9	9.3	0.76	1151.1	1154.0	2.9	0.25
7	234	599	1237.0	1205.0	-32.0	-2.59	1162.5	1126.4	-36.1	-3.1
8	236	601	1168.0	1171.8	3.8	0.33	1126.0	1111.2	-14.8	-1.31
9	241	606	1171.7	1166.0	-5.7	-0.49	1152.4	1167.1	14.7	1.28
10	352	717	1200.5	1231.9	31.4	2.62	1173.8	1185.5	11.7	1.0
11	356	721	1267.6	1247.9	-19.7	-1.55	1201.7	1211.3	9.6	0.8
12	258	623	1260.3	1272.5	12.2	0.97	1180.4	1182.0	1.6	0.14
13	263	628	1222.9	1236.1	13.2	1.08	1203.1	1181.5	-21.6	-1.8
14	340	705	1253.0	1232.5	-20.5	-1.64	1156.0	1147.1	-8.9	-0.77
15	315	680	1194.7	1192.6	-2.1	-0.18	1187.1	1186.3	-0.8	-0.07
16	512	877	1206.2	1204.1	-2.1	-0.17	1155.1	1155.6	0.5	0.04

Chapter 5

DISCUSSION

5.1 Discussion of Thesis Results

pQCT technology allows for the measurement of volumetric bone mineral density (mg/cm^3), differentiation between trabecular and cortical components of bone, examination of changes and redistributions of bone mineral, and quantification of cross-sectional geometric properties of bone tissue at peripheral skeletal sites. This thesis assessed pQCT measurements of cortical and trabecular bone density and geometry at the non-dominant radius and tibia in postmenopausal women, with a specific focus on the effects of menopause and breast cancer therapy on musculoskeletal health. The objectives of this research were as follows: (*i*) to determine if the indices of bone density and geometry, as well as muscle and fat area, can be measured reliably at the radius and tibia in healthy pre- and postmenopausal women, and to assess pQCT differences in bone density, content, and geometry between pre- and postmenopausal women; (*ii*) to examine the level of osteoporosis knowledge in a cohort of postmenopausal women and compare this with the knowledge scores in women with a history of breast carcinoma; (*iii*) to evaluate, via pQCT, the cross-sectional values for radial and tibial cortical and trabecular bone as well as calf muscle, and fat cross-sectional area in healthy postmenopausal women and women with a history of breast carcinoma; (iv) to compare bone density and content measurements conducted via DXA with those by pQCT; and (*v*) to assess the degree of change in cortical and trabecular bone measurement parameters in breast cancer patients after 12 and 24 months of treatment with Anastrozole.

5.1.1 Reproducibility of pQCT Measurements

pQCT was introduced over 30 years ago for imaging of the radius; since that time, the original design has undergone numerous modifications.(Ruegsegger *et al.,* 1976; Schneider &

Borner, 1991) Most often, pQCT has been applied to measurements at the radius, and reproducibility values for this site have been assessed for many pQCT scanners. Only recently have pQCT scanners been utilized for measurements at the tibia.(Braun *et al.,* 1998; Sievanen *et al.,* 1998) The XCT-2000 pQCT scanner was developed by Stratec Medizintechnik (Pforzheim, Germany), and the only precision studies of tibia measurements that have been conducted with an XCT-2000 device were in young children at the distal tibia(Binkley & Specker, 2000) and in young adults with tibial shaft fracture.(Findlay *et al.,* 2002) Measurement precision is dependent upon a number of factors which include the *in vivo* nature of the measurement, the population of interest, the device utilized, and operator technique. Therefore, an assessment of the reproducibility of the XCT-2000 device at the tibia adds valuable data to the area of study concerning pQCT imaging. This is due to the fact that the proximal hip, which is a common site of fracture in osteoporotic women, has a similar mechanical loading history to the tibia.

The objectives of the first study of this thesis were to evaluate pQCT variables for the radius and tibia in pre- and postmenopausal women, and to assess the *in vivo* reproducibility of density, content, and geometry measurements using the XCT-2000 pQCT scanner. It was anticipated that pQCT measurements at the tibia would complement measurements at the radius. Any differences between pre- and postmenopausal women with respect to bone geometry at the diaphyseal radius and tibia due to the loading differences of these limbs was investigated. Furthermore, assessment of the *in vivo* reproducibility of the pQCT technique for muscle and subcutaneous fat determination at the mid-calf was conducted. This *in vivo* evaluation demonstrates that the XCT-2000 pQCT scanner provides efficient precision, not

only for the upper limb bones for which it was primarily designed, but also for the tibia. The distal tibia gave the most favorable reproducibility for TOT_DEN (1.5%) and TRAB_DEN (1.6%), while the diaphyseal tibia was the most reproducible site for CRT_DEN (0.3%). This may be a consequence of the fact that it is easier for patients to keep the tibia immobile during a scan. At the radius, small movements that can be imperceptible to patients may significantly affect precision. Furthermore, given the strong interrelationship between muscle force and bone strength, quantification of muscle area changes may also be valuable in the diagnosis of bone disorders. In this study, one patient could not complete the muscle area measurement at the 66% tibia site due to the limited size of the aperture. A similar problem was encountered by others at the proximal 10% tibia site.(Findlay *et al.,* 2002) However, no significant differences were detected in muscle area between pre- and postmenopausal women.

Studies have reported CV values for the XCT-900 and XCT-960 scanners of less than 3% for total BMD and less than 2% for trabecular BMD at the radius.(Augat *et al.,* 1998b; Butz *et al.,* 1994; Grampp *et al.,* 1995; Guglielmi *et al.,* 2000; Martin *et al.,* 1999; Takada *et al.,* 1996) However, most of these studies did not report the characteristics of the subjects involved. Boonen *et al.* measured *in vivo* precision at the distal radius using the XCT-900 in 129 healthy female subjects who were aged 70 to 87 years.(Boonen *et al.,* 1997a) In this population, they reported CV precision errors of 2.4%, 1.9% and 2.2% for total, trabecular and cortical densities, respectively. With few exceptions,(Braun *et al.,* 1998; Sievanen *et al.,* 1998) pQCT has been almost exclusively applied at the distal radius,(Augat *et al.,* 1998b; Augat *et al.,* 1998a; Guglielmi *et al.,* 1997; Nijs *et al.,* 1998) and only very recently have new

pQCT scanners been developed for measuring the tibia and femur. The necessity of characterizing the precision error of this technique for the specific population of concern in any given study was demonstrated by Grampp *et al.*(Grampp *et al.,* 1995) They used the XCT-960 to measure radial pQCT reproducibly in healthy pre- and postmenopausal women and in osteoporotic subjects and found that the precision error was higher in the osteoporotic group than the healthy subjects for TOT_DEN and TRAB_DEN.(Grampp *et al.,* 1995) Sievanen *et al* used the most recent version of pQCT scanners (XCT 3000) to measure *rms*CV values at the distal radius in 19 volunteers (12 men and 7 women), and at the distal tibia in 36 volunteers (15 men, 21 women), and found values similar to those determined in the study presented here utilizing the XCT-2000 scanner.(Sievanen *et al.,* 1998) Yet, the subjects in the current study were pooled pre- and postmenopausal patients, which may have increased the variability in the measurements given the age-related changes which occur in bone density and structure.

It is noteworthy that the mean value for TRAB_DEN of the weight bearing distal tibia for all persons in this study (221 mg/cm^3) was significantly greater ($p=0.034$) than that of the non-weight bearing distal radius (197 mg/cm^3). Also, the mean values for CRT_DEN of the diaphyseal radius and tibia were significantly different ($p<0.001$). This is in contrast with a report(Sievanen *et al.* 1998) in which the mean TRAB_DEN at the radius and tibia were found to be 237 mg/cm^3 and 235 mg/cm^3 respectively. This may be due to the fact that the study by Sievanen *et al,*(Sievanen *et al.* 1998) included both men and women in the analysis of TRAB_DEN, whereas this study involved women only. At the distal radius, none of the variables were significantly lower in the postmenopausal women as compared with the

premenopausal women. Intriguingly, at the distal tibia, TOT_DEN and TRAB_DEN were significantly lower in postmenopausal women. Similar results were observed in a larger study conducted by Boutroy *et al.*,(Boutroy *et al.*, 2005) in which a high-resolution HR-pQCT device was utilized. Boutroy *et al.* found that all parameters except for TOT_A were significantly different between pre- and postmenopausal women at the distal tibia. Moreover, using the Densiscan 1000 (ScancoMedical, Zurich, Switzerland) in a similar population, Tsurusaki *et al.* showed that the rate of diaphyseal tibial vBMD loss was significantly greater than radial vBMD loss in postmenopausal women.(Tsurusaki *et al.*, 2000) In an *in situ* study by Groll *et al.*, the correlation between trabecular bone at the tibia and the femur was found to be higher than the correlation between the radius and the femur, using an XCT-3000 pQCT scanner.(Groll *et al.*, 1999) Clearly, for scanners which cannot measure femoral bone density, a closer prediction of femoral bone properties is available from tibia measurements rather than radius measurements. The results of this substudy suggest that since the *in vivo* *rms*CV values of the XCT-2000 pQCT device were excellent, particularly at the tibial sites, bone mineral measurements at the distal tibia may be a useful tool in the evaluation of age-related bone loss.

5.1.2 Osteoporosis Knowledge

Scientific progress and new investigative and therapeutic methods for the diagnosis and treatment of osteoporosis have heightened awareness concerning this condition, both within the medical profession and throughout the general population. Currently, considerable effort is directed towards patient education – formally, as circulated in information leaflets and disseminated through patient consultation with physicians, and informally, via the popular

media. The World Health Organization has defined osteoporosis in terms of bone mass that is more than 2.5 SD below the mean measurement for peak bone mass in healthy young adults.(Cummings & Black, 1995) Based on this criterion, it has been estimated that 13-18% of women aged 50 and over have osteoporosis,(Wolf *et al.,* 2000) and for those over the age of 80, the incidence rises to 70%.(Melton, 1995) One serious outcome of osteoporosis is hip fracture, and the number of hip fractures world-wide is projected to increase from 1.7 million in 1990 to 6.3 million in 2050.(Johnell, 1997) The annual cost of care associated with hip fracture in Canada has been estimated at $650 million.(Wiktorowicz *et al.,* 2001) Equally alarming figures regarding the prevalence, consequences, and costs of osteoporosis have been reported for Australia, the United States and Great Britain. Furthermore, a history of prior fracture has been shown to be an independent predictor of subsequent fractures at any site.(Kanis *et al.,* 2004)

The costs incurred with fragility fractures are not only measurable in health care dollars, but also in terms of physical and psychological effects involving functional limitations, changes in bodily stature, chronic pain, decreased quality of life, reduced productivity, and increased mortality.(Adachi *et al.,* 2001; Aharonoff *et al.,* 1997; Magaziner *et al.,* 1990) Among postmenopausal women, it has been estimated that one in six will suffer a hip fracture.(Grady *et al.,* 1992) Twenty percent of individuals who experience a hip fracture will die from related complications within the first year post-fracture(Keene *et al.,* 1993; Lorrain *et al.,* 2003; Meine *et al.,* 1993; Ray *et al.,* 1990; Sernbo & Johnell, 1993; White *et al.,* 1987) and 60% in the first six years post-fracture.(Jalovaara & Virkkunen, 1991) Moreover, the absence of symptoms prior to fracture can hinder diagnosis and, hence, curb the effectiveness of

therapeutic intervention. While the onset of osteoporosis can provoke serious consequences, it is widely recognized that this disease is preventable, and numerous studies have found clear associations between several health behaviors and a decreased risk of osteoporosis. Thus, encouraging primary prevention and early detection are of fundamental importance; once established, osteoporosis is difficult to reverse.

Patient knowledge questionnaires, such as the FOOQ, can assist clinicians and health educators in identifying those individuals in need of educational interventions. The FOOQ may also provide a valid and reliable approach to evaluating the effectiveness of education programs for individuals and at-risk groups. In the present study, it is encouraging that over half (53%) of the patients identified that they had spoken to a health care professional about osteoporosis, primarily their family doctor (83%). It is likely that these discussions translated into higher scores on the FOOQ than would otherwise have been the case. The breast cancer subjects had met with their oncologist prior to entering the study, however, the focus of the oncologist on issues related to osteoporosis in these women is obviously secondary to the fundamental concerns of the cancer treatment. Nonetheless, all of the breast cancer patients had consulted with a rheumatologist and had also undergone a BMD scan prior to their involvement in this study. Notwithstanding, the mean score (SD) for the breast cancer subjects was 13 (3). The results indicate, rather surprisingly, that women's knowledge about osteoporosis is deficient even among this group of women who had contact with bone-health medical professionals on multiple occasions. Moreover, these postmenopausal survivors of breast cancer had demonstrated several other risk factors for the development of osteoporosis. For example, almost half the subjects (44%) had a family history of osteoporosis; 48% had

been smokers at one stage in their lives; and all were Caucasian. Additionally, the majority of women in this study spent very little time engaged in weight-bearing activities, with significantly more time spent engaged in moderate to light activities. One of the major knowledge disparities among study participants was found in the identification of risk factors. Without this knowledge women lack a framework for determining their own risk of developing the disease, and are unlikely to have the perspicacity to request diagnostic tests and initiate appropriate preventive habits before a first fracture occurs.

Optimizing long-term lifestyle behaviors, concerning calcium and vitamin D intake (diet and/or supplements), smoking, alcohol intake, and physical activity, may influence prevention or development of osteoporosis.(Nelson *et al.,* 1994; Suleiman *et al.,* 1997; Wolff *et al.,* 1999) With respect to exercise, 99% of the women in this study were misinformed or incorrect that walking has a great positive effect on bone health. Of course, walking is beneficial for promoting fitness, but it is important to stress weight-bearing activity as the best activity for increasing BMD scores.(Kronhed & Möller, 1998) Progressive strength/weight training has known benefits for countering bone loss in postmenopausal women.(Heinonen *et al.,* 1996; Kerr *et al.,* 1996; Nelson *et al.,* 1994; Picard *et al.,* 2000; Pruitt *et al.,* 1995; Snow-Harter *et al.,* 1992) When considering physical activity in this population, the data suggest that on average these women were not participating at all in daily weight-bearing activities. Intervention trials looking at the effect of weight-bearing physical activity on bone mass have found positive results only with high-intensity exertion.(Bassey & Ramsdale, 1994; Dalsky *et al.,* 1988; Heinonen *et al.,* 1996; Nelson *et al.,* 1994; Snow-Harter *et al.,* 1992) Studies looking at low-intensity exercise found no effect on BMD.(Lord *et al.,*

1996) As the majority of total weight-bearing physical activity of this group was accounted for in housework, brisk walking, golfing, and gardening, it is clear that most women were not getting enough weight-bearing exercise of sufficient intensity to improve bone mass. Furthermore, as compared with the healthy postmenopausal women, even fewer of the breast cancer patients stated that they participated in any form of strenuous sport or vigorous work that could be included as a weight-bearing activity.

In regards to dietary requirements for calcium, 21% either did not know or were misinformed regarding whether a lifetime of low calcium and vitamin D intake increases the risk of osteoporosis, and the same percentage of subjects did not know how much calcium women need per day after menopause. This is likely reflected by the fact that 21% of the participants were not taking any dietary supplementation with calcium and 33% were not taking vitamin D. Furthermore, 47% of the participants did not know if alcoholism is linked to osteoporosis. In the total cohort, 34% consumed some form of alcoholic beverage on a daily basis. This finding suggests that additional research in this area may be beneficial, as abuse of alcohol has been shown to be an important risk factor for osteoporosis and fractures.(Laitinen & Välimäki, 1991; Rico, 1990) In addition, 45% did not know that early menopause is a risk factor for osteoporosis and 59% did not know that replacing hormones after menopause can slow down bone loss.

It is a promising indication that a substantial proportion of all respondents had stopped practicing behaviors believed by many experts to exacerbate the onset or progression of osteoporosis. Somewhat more than half (55%) of the women reported never smoking, and

39% (HCS) and 33% (BCS) of the smokers in each group reported having quit the habit. In addition, the majority of women participated in some form of physical activity on a regular basis. Also, 100% of the breast cancer patients reported taking calcium supplements and 96% reported taking vitamin D. Improved public education may lead to preventative behaviors and, ultimately, a decreased incidence of osteoporosis. Therefore efforts should be invested in developing theoretically based studies, aimed mainly at identifying what areas of knowledge should be expanded in order to promote behavioral changes. This could be achieved by incorporating more knowledge about the disease into health promotion programs.

In order to evaluate patient awareness concerning osteoporosis, it is necessary to examine how effective professionals involved in its prevention and treatment are at communicating preventative advice to those most likely to be affected by the disease.(Cranney *et al.,* 2002) Extensive literature exists on the many potential strategies to prevent osteoporosis and to treat established disease, but this has not necessarily been translated into clinical practice. In a similar study, the average score on the initial version of the FOOQ was 16 (SD= 4.87; range = 1 to 25) with a sample size of 247 women.(Ailinger & Emerson, 1998) The authors indicated that the majority of these women did not have adequate knowledge about osteoporosis or associated risk factors and preventative behaviors.(Ailinger & Emerson, 1998)

Additional studies have shown that many women do not have a clear understanding of osteoporosis and the knowledge they do have may not be linked to behavior.(Ailinger & Emerson, 1998; Magnus *et al.,* 1996; Taggart & Connor, 1995) However, on a positive note,

women who reported that they had received previous information about osteoporosis also demonstrated higher knowledge in their test scores.(Ailinger & Emerson, 1998) This finding suggests that education about this preventable disease is advantageous. Most notably, there is evidence regarding the association between decreased risk of osteoporosis and participation in physical activity, cessation of smoking, and adequate dietary intake of calcium and vitamin D.(NIH Consensus Development Panel on Osteoporosis Prevention, 2001; Wolf *et al.,* 2000) Modest, but consistent results have also been reported, showing a relationship between abuse of alcohol and caffeine and low bone mass.(Høidrup *et al.,* 1999; Meyer *et al.,* 1997) Cross-sectional studies have varied in their findings with regard to the association between levels of osteoporosis knowledge and osteoporosis preventative behaviors.(Kasper *et al.,* 1994; Satterfield *et al.,* 2000; Taggart & Connor, 1995; Terrio & Auld, 2002; Wallace, 2002) A number of cross-sectional studies that have examined the relationship between knowledge and participation in health related behaviors have shown significant, favorable associations,(Ali & Bennett, 1992; Werner, 2003; Werner *et al.,* 2003) whereas the study by Wallace did not find such a relationship.(Wallace, 2002) An intervention study found that self-reported lifestyle behaviors improved in premenopausal women after they had received information about osteoporosis while having a bone mineral density test.(Jamal *et al.,* 1999) Prospective studies are likewise inconclusive, with some suggesting concurrent improvements in osteoporosis knowledge and preventative behavior,(Curry *et al.,* 2002) while others show no resultant impact on behavior from an increased awareness of tactics for disease prevention.(Blalock *et al.,* 2002; Sedlak *et al.,* 2000) There is evidence that osteoporosis knowledge is one contributor to osteoporosis preventive behaviors, although the progression from knowledge to action has not, as yet, been fully elucidated.

5.1.3 pQCT Assessment: Women with a History of Breast Carcinoma

The primary aim of this thesis was to apply pQCT technology in an assessment of skeletal density, content, and geometry at the radius and tibia, with a focus on the effects of menopause and breast cancer therapy on trabecular and cortical bone. The variables that were found to be significantly lower in the breast cancer subjects were TOT_DEN and TOT_CNT at the 4% radius; TOT_DEN at the 4% tibia; CRT_DEN, TOT_CNT, and CRT_CNT at the 20% radius; and CRT_DEN at the 38% tibia. The mean TOT_A of the distal tibia was slightly higher (1022 mm^2 in breast cancer subjects and 986 mm^2 in healthy controls), although not significantly higher. In addition, the total cross-sectional areas of muscle, subcutaneous fat, and bone areas at the 66% tibia site were lower in the breast cancer subjects, but not to a significant degree. When the variables that were significantly different between the two groups were investigated further, regression analysis revealed several notable observations. At the distal radius, the largest variation in the TOT_CNT data set could be accounted for by the TOA (R^2=5.3% TOT_DEN, R^2=12.9% TOT_CNT); BMI positively accounted for approximately 10% of the variability of the data sets; R^2=9.6% for TOT_DEN and R^2=9.9% for TOT_CNT. At the distal tibia, approximately 16% of the variation in TOT_DEN (R^2=16.4%) can be explained by BMI, and the TOA (R^2=4.3%), with the subjects age (R^2=0.1%), AAM (R^2=0.9%), YSM (R^2=0.5%), NOC (R^2=3.3%) accounting for a very small percentage of variability in TOT_DEN. At the diaphyseal radius, the TOA (R^2=19.5% for CRT_DEN, R^2=11.2% for CRT_CNT and R^2=11.1% for TOT_CNT) and age of the study participants (R^2=16.1% for CRT_DEN, R^2=17.9% for CRT_CNT and R^2=13.7% for TOT_CNT) accounted for the highest percentage of variability among anthropometric factors in the data sets. And finally, at the diaphyseal tibia, univariate regression analysis

demonstrate that less than 6% of the variation in CRT_DEN can be accounted for by the age of the study participants (R^2=5%) and the YSM (R^2=5.5%). The variation due to BMI (R^2=0.2%), AAM (R^2=0.4%), and NOC (R^2=2.8%) were all very low. TOA contributed to the highest variation in the data set (R^2=15.1%). Interestingly, of the primarily cortical bone sites, the 20% radius and 38% tibia, TOT_CNT (r=-0.32 and r=-0.36 respectively) and CRT_CNT (r=-0.31) measurements showed a significant, inverse association with the number of biological children in the sample population.

When looking at the longitudinal change in the breast cancer subjects after 12 and 24 months of Anastrozole therapy, at the distal radius, the mean values for TOT_CNT, TRAB_CNT, TOT_DEN and TRAB_DEN appeared to remain relatively constant over the 2 year period; there was no statistically significant change in the mean for any of these variables ($p>0.05$). The results were similar at the 4% distal tibia, with no statistically significant difference in the mean values for total or trabecular bone density and content. However, at the 20% diaphyseal radius, there was a significant decrease in CRT_DEN ($p=0.025$) during the 2 year follow-up. Also at the 38% diaphyseal tibia, the decrease in CRT_DEN closely approached significance at $p=0.051$. In addition, when the data from the 5 subjects with 2 year follow-up measurements was further analyzed by linear regression to determine the percentage change per year, the subject taking a bisphosphonate experienced a positive increase in CRT_DEN of 1.1%, and the other four experienced a decrease. The same pattern was reflected at the tibia, in which the subject that was taking a bisphosphonate experienced the smallest decrease of CRT_DEN at -0.4%. Finally, when sixteen breast cancer subjects undergoing Anastrozole therapy were measured via pQCT at two time points during treatment, a significant positive

association (R^2=37.7%) between the percentage change in CRT_DEN at the radius and tibia (r=0.61, p=0.01) was determined. Therefore, from these data, time on Anastrozole primarily negatively affects cortical bone in these breast cancer subjects, with increasing age and years since menopause exerting a further negative effect on bone variables at the diaphyseal sites, and increasing BMI having a positive effect on bone variables at the distal sites of the radius and tibia.

Skeletal sites high in trabecular bone content, such as the ultradistal radius, have generally been identified as the optimal sites for BMD measurement. Despite the excellent *rms*CV values for cortical bone density measurements at the diaphyseal radius and tibia, cortical bone has not often been considered a good variable for characterizing skeletal status. Trabecular bone has a large area-to-volume ratio, is metabolically active tissue, and exhibits large inter-individual variability in density. This type of bone can therefore demonstrate marked changes between various skeletal disorders and show appreciable early responses to various treatments. The conventional view of osteoporosis entails that the bone fragility is primarily due to a disease of trabecular bone. It has also been shown that the density of a purely cortical bone remains virtually constant regardless of bone size, age or even the degree of osteoporosis.(Ruegsegger *et al.,* 1991b) However, Crabtree *et al.* demonstrated that when comparing female hip fracture cases and controls, the differences were primarily in cortical rather than trabecular bone.(Crabtree *et al.,* 2001) Furthermore, studies have shown that cortical and trabecular bone loss is homogeneous in healthy women,(Nijs *et al.,* 1998; Wapniarz *et al.,* 1997) whereas others report a significant age-dependent loss of cortical but not trabecular bone.(Boonen *et al.,* 1997b) Some cross-sectional findings suggest that

trabecular bone density begins to decrease before middle age and proceeds linearly whereas cortical bone density begins to decrease at menopause.(Riggs *et al.,* 2004; Russo *et al.,* 2003) Qin *et al.* demonstrate that both trabecular and cortical bone loss are greater within the first 3 menopausal years compared to later postmenopausal years.(Hangartner & Gilsanz, 1996; Qin *et al.,* 2002)

It is important to note, especially in the case of thin cortices, that the partial volume effect inherent to a tomographic measurement can result in underestimated cortical values.(Augat *et al.,* 1998c; Hangartner & Gilsanz, 1996; Prevrhal *et al.,* 1999) The risk of fracture and the mechanical failure loads of the proximal femur and distal radius have been shown to depend not only on the total bone mass, but also more specifically on the thickness and geometric properties of the cortical shell.(Augat *et al.,* 1996) During aging the cortical cross-sectional area declines while the total bone area increases owing to periosteal apposition.(Ahlborg *et al.,* 2003; Russo *et al.,* 2003; Seeman, 1997) Thus, expansion of the outer diameter partially preserves the bone's ability to resist bending and torsion, although there is a net loss in the amount of bone mineral with aging. Interestingly, in this thesis, despite significant differences in CRT_DEN between the two cohorts, the PMR and SSI were not significantly different at the diaphyseal radius or tibia between the two cohorts. SSI is calculated using the cortical volumetric density and the cross-sectional moment of inertia. Therefore, this is an important validated biomechanical strength bone parameter that is related to breaking force.(Ferretti *et al.,* 1996) More detailed study is required to confirm the significance of these findings in the context of changes in bone health which occur during the postmenopausal aging process as well as in women that have undergone various therapy

regimens for breast cancer.

As the vast majority of fractures of the distal radius (95%) occur within 1.6 and 3.0 cm of the tip of the radial styloid process,(Eastell *et al.,* 1989) an intact trabecular network also appears to be vital for maintaining maximum bone strength.(Borah *et al.,* 2001) Given the common fracture occurrence at this site among postmenopausal women,(Black *et al.,* 1992) a fracture in a bone high in trabecular content is considered to be one of the earliest clinical manifestations of primary (type I) osteoporosis, and an early recognition of this manifestation allows the time for preventive measures to have an effect.(Earnshaw *et al.,* 1998) A prior forearm fracture is associated with about a twofold increase in the likelihood of a subsequent fracture.(Cuddihy *et al.,* 1999; Finsen & Benum, 1987; Gay, 1974; Lauritzen *et al.,* 1993; Mallmin *et al.,* 1993) One of the first prospective studies by Gärdsell and colleagues(Gärdsell *et al.,* 1989) found that measurements of bone mass at the radius strongly related to the risk of subsequent fractures in women who were <70 years old at the time of the measurement. An updated analysis, reported that radius BMC was also associated with risk for subsequent fractures in women age 70-80 years, but not consistently so in women >80 years(Gardsell *et al.,* 1993). Melton *et al.*(Melton *et al.,* 1993) found similarly strong relationships between the risk of hip fracture and bone density measured in the distal radius (relative risk = 2.6; 95% confidence interval 1.2-5.4). Cummings and colleagues (Cummings *et al.,* 1993) demonstrated that bone mass measured in any of the regions of the proximal femur was more strongly associated with the subsequent risk of hip fracture than bone mass measured in the radius, calcaneus or spine, and they also reported that BMD is more strongly associated with the risk of hip fracture than BMC(Cummings *et al.,* 1994). From these

reports, it is not consistently clear which site is superior for predicting spine, hip, or other types of fractures. Moreover, because bone turnover occurs at different rates in different bones, BMD at one site does not necessarily indicate a similar level of bone mass at another site. As such, a patient may have a low BMD reading at one site but not at another. The spine and hip are the skeletal sites conventionally used for diagnosing osteoporosis, with diagnosis determined by the lower BMD measurement. Great differences in turnover between different bones and regions within bones obviously place constraints on the capacity of biochemical indices to capture the behavior of the entire skeleton from a single bone site. Until recently, most researchers have relied on DXA to describe bone characteristics; aBMD is useful for measuring the planar projection of bone mass, but it only provides a two-dimensional view of a three-dimensional structure. Although aBMD provides a 'surrogate measure of bone strength' it overlooks the structural features of cortical and trabecular bone. DXA is unable to assess geometric adaptations because of its planar nature. That is, a larger bone will be seen as having more density, and this can hide structural differences that may exist.

pQCT provides measures of the vBMD and site-specific geometric properties; pQCT results can also be used to generate a bone strength index that reflects the combined strength of both trabecular and cortical bone to resist bending or torsion. In examining the difference between subjects for each bone variable, certain patterns emerge. For example, it appears that total and cortical bone content measurements are generally reduced in women with a history of breast carcinoma. Furthermore, the radius appears to be the site which shows the greatest degree of difference between the two cohorts. In addition, when examining the relationship

between the DXA measurements and pQCT scores in the breast cancer subjects, the two sites utilized for the diagnosis of osteoporosis, namely the lumbar spine BMD and the femoral neck BMD, did not correlate with many of the sites measured by pQCT that were found to be significantly different between the two populations. Of the significantly different sites, lumbar spine BMD was correlated with only CRT_DEN and CRT_CNT at the 20% radius and femoral neck BMD was only correlated with 4% tibia TOT_DEN. Interestingly, the 4% distal tibia TOT_CNT significantly correlated with all the DXA measurement sites, including the lumbar spine, total hip, non-dominant arm and leg and total body measurements. Furthermore, the TOT_CNT at the 4% radius significantly ($p<0.01$) correlated with all the BMC measurements assessed via DXA; this included the lumbar spine BMC, total hip BMC, non-dominant arm and leg BMC, and total body BMC. Perhaps assessment of fracture risk would benefit from the analysis of multiple bone sites, including an evaluation of cortical density and content via pQCT, rather than DXA BMD thresholds alone. (Kanis, 2002)

In this thesis, serum vitamin D levels were only significantly correlated with CRT_DEN at the 38% tibia (r=0.54; p=0.007) in the breast cancer subjects. As these women were all prescribed vitamin D and calcium, it is likely that their serum levels would be higher than those of the healthy controls. Supplementation with vitamin D reduces rates of bone loss in older adults (Ooms *et al.* 2009) as does supplementation with both calcium and vitamin D together(Dawson-Hughes *et al.,* 1997). The higher levels of calcium and vitamin D need to be maintained(Dawson-Hughes *et al.,* 2000) in order to sustain the reduced turnover rate and higher bone mass. Additionally, the effect of supplemental vitamin D on fracture incidence has been examined in several large trials. Findings suggest that annual subcutaneous

injections of vitamin D lower all clinical fractures(Heikinheimo *et al.,* 1992), but others have found no effect of vitamin D in doses of 300 or 400 IU per day on fracture rates at any skeletal site(Komulainen *et al.,* 1998; Lips *et al.,* 1996; Meyer *et al.,* 2002). However, supplementation with calcium and vitamin D together (in doses of 500-1200 mg of calcium and 700-800 IU of vitamin D) significantly reduced all clinical fractures including hip fractures rates(Chapuy *et al.,* 1992; Chapuy *et al.,* 2002; Dawson-Hughes *et al.,* 1997). The significance of the correlation between CRT_DEN and vitamin D levels remains to be determined in the context of the long-term effects of such supplementation on fracture incidence in breast cancer subjects.

The association between BMD and incidence of breast cancer has been investigated in a number of studies.(Bruning *et al.,* 1990; Cauley *et al.,* 1996; Kuller *et al.,* 1997; Olsson & Hägglund, 1992; Persson *et al.,* 1994; Zhang *et al.,* 1997) In a 12-year follow up study, Olson and Hägglund reported a 46% lower risk of breast cancer in women who sustained forearm fracture.(Olsson & Hägglund, 1992) Similarly, Persson *et al.*, in a large population based cohort study, found a 16% lower risk for breast cancer in women who had sustained a hip fracture.(Persson *et al.,* 1994) Furthermore, Cummings *et al.*, showed a 62% lower risk of breast cancer in women with vertebral fractures compared with controls, even after adjustment for BMD. However, others have failed to show this association.(Adami *et al.,* 1990) A population based cohort study by Adami *et al.* found no differences in the incidence of first hip fracture in a varied sample of women with breast cancer. These findings have only limited validity, because results from women with breast cancer were not compared with those from a control group matched for possible confounding variables such as age, body

mass index and cumulative exposure to estrogen.(Kanis *et al.,* 1999) The results of the Marburg breast cancer and osteoporosis trial(Hadji *et al.,* 2007) are consistent with the observation that women with breast cancer have higher bone mass and therefore a lower risk for osteoporosis and fracture than healthy controls. It is possible that the observed association may be related to other hormonal factors such as progestins, androgens, prolactin, growth hormone, or even insulin, all of which have been shown to be directly related to BMD and to the risk of breast cancer.(Helzlsouer *et al.,* 1994) Although studies have suggested a correlation between high BMD and increased risk of breast cancer,(Cauley *et al.,* 1996; Zhang *et al.,* 1997) there is also evidence indicating that breast cancer survivors are at an increased risk of accelerated bone loss,(Bruning *et al.,* 1990; Greep *et al.,* 2003) which could lead to a higher risk of fracture.(Chen *et al.,* 2005; Kanis *et al.,* 1999) Breast cancer survivors are a growing population; given that breast cancer has become a chronic disease for many women, preventing bone loss may play a significant role in reducing co-morbidity and in improving health-related quality of life in the long-term.

Absorptiometry and other diagnostic tools, together with recently introduced clinical approaches to osteoporosis, have opened up new possibilities for the prompt treatment of osteoporotic fractures, the prevention of further fractures and, above all, primary prevention of fractures. Underlying malignancy, antineoplastic treatments, and the direct toxic effects of chemotherapy agents on the cells involved in bone formation can all contribute to an accelerated rate of bone turnover among breast cancer survivors, as do the cancer-associated co-morbid conditions, including malnutrition, loss of muscle mass, and immobilization. In addition, ovarian failure and early menopause induced by adjuvant chemotherapy,

oophrectomy in premenopausal women, and restriction of hormone replacement therapy after breast cancer diagnosis can lead to increased bone loss. Cancer prognosis itself, along with lifestyle changes after cancer diagnosis, may also contribute to the low BMD among breast cancer survivors. Adjuvant therapies for breast cancer survivors now often involve AIs. The American Society of Clinical oncology supports yearly DXA scans for postmenopausal breast cancer survivors at high risk, which includes patients who are taking AIs. In this thesis, the breast cancer subjects showed a trend towards decreasing cortical bone density and content, particularly at the 20% diaphyseal radius; at this site, the mean CRT_DEN was significantly ($p=0.025$) reduced from baseline values. Whether the guidelines for annual BMD testing will increase awareness among medical oncologists and demonstrate a positive impact upon the diagnosis and prevention of osteoporosis in breast cancer survivors remains to be seen.

5.2 Study Limitations

When utilizing pQCT imaging technology, close attention must be paid to potential sources of imprecision including soft tissue thickness, the amount of marrow fat, beam hardening, inconsistencies in the alignment of the target bones with respect to orientation of the tomographic slice, and subject comfort so that possible movement artifacts can be minimized.(Grampp *et al.,* 1995) For this thesis, verification of correct subject positioning relied on visual inspection of limb alignment. This is susceptible to some variability as any small changes in the reference line could result in considerable differences in the measured parameters. At the distal sites of either the radius or the tibia, this is particularly a concern because cross-sectional geometry changes rapidly along the longitudinal axis of the given bone. In addition, to minimize variation which may be associated with the operator, all

measurements were obtained by two people trained to measure in the same manner. This is advantageous as simple factors such as determining limb length measurements can be problematic if not fully standardized between operators.

In this thesis, the study sample populations were not representative of the total population; random selection did not occur, with women self-selecting to enroll in the study. The study was exploratory and based on a sample of convenience from one geographical area; it consisted primarily of middle-class, English-speaking women who were relatively well-educated and had access to both professional and media sources of health information. The recruitment strategies employed for the breast cancer patients were intended to ensure the representation of postmenopausal women. Recruiting participants directly from clinics might have biased the sample by including those participants with relatively high health beliefs and those better motivated to co-operate with healthcare providers. Furthermore, women with better knowledge of osteoporosis may have been more willing to participate in this study, consequently achieving higher knowledge scores. Therefore, the actual level of knowledge in the general population is likely to be even lower than that assessed in the present study. In addition, participant self-reports were used to measure adherence with exercise regimes. Although self-reports are generally more accurate than estimates from physicians or family members, this form of reporting often results in overestimated levels of participant adherence to beneficial behaviors.(O'Brien *et al.,* 1992) The information provided by the participants regarding their baseline characteristics (such as demographic, reproductive and fracture history, medication usage and health status data) relied on subject recall of this information.

However, the influence of a recall bias on both participant groups should be equivalent and therefore negligible.

DXA measurements of spine and hip in the healthy control population were not included in the cross-sectional comparison between women with and without breast cancer; therefore, a comparison of the effects of breast cancer and its associated therapy on these clinically important skeletal sites could not be evaluated. Furthermore, the study design only allowed for speculation about the temporal nature of any bone changes in the control subjects. The participant inclusion and exclusion criteria were used to obtain a relatively homogeneous sample. Because the sample sizes were relatively small, other clinically relevant questions could not be examined, such as serum estradiol levels during menstrual cycles, rates of anovulatory cycles, corpus luteum insufficiencies, abnormal bleeding, or an increased or decreased cycle length. These factors may lead to significant differences in exposure to estrogen and the extent to which these women reached peak bone mass before menopause. Ultimately this may have contributed to the noted differences in cortical and total bone density and content. These important aspects can only be addressed in a large, long-term, prospective trial with random assignment to a treatment or comparison group.

5.3 Recommendations for Further Research

The financial, social, and personal burdens that osteoporosis imposes can often be minimized through prevention. A motivating aspect of this disease is that its severity may be reduced by optimizing peak bone mass in youth, maintaining bone mass in adulthood, and minimizing bone loss in later years. Accurate knowledge of risk factors may facilitate women's

attentiveness to their relative degree of vulnerability for osteoporosis, and by extension, encourage help-seeking behaviors, while promoting early detection of bone-health changes. Indeed, research has shown that a woman's willingness to take appropriate preventive action depends in large part on her level of knowledge about factors which may increase or decrease her chances of developing osteoporosis.(Rubin & Cummings, 1992) The etiology of osteoporosis is multi-factorial, which can lead to hazardous misunderstandings among patients at risk, concerning the efficacy of preventative behaviors. More insidiously, osteoporosis develops silently and often goes undiagnosed until there is a fracture. Proposed risk factors include; heredity, estrogen deficiency, caffeine, alcohol and tobacco use, inadequate calcium intake, vitamin D deficiency, sedentary lifestyle, deficient weight-bearing exercise, and low BMI. Several of these factors may be addressed through intervention, but regrettably, many women are unaware of their own capacity to combat the onset of this disease.(Drugay, 1997) Women need to accurately discern the risk factors associated with osteoporosis in order to engage in self-care strategies and make appropriate steps in preventative behavior, alongside informed choices about available treatments. Given that breast cancer survivors are a growing population, preventing bone loss in these women may play a significant role in health-related quality of life after breast cancer diagnosis. Due to the specific risk factors for breast cancer patients, an even more extensive evaluation of osteoporosis knowledge and effective prevention strategies is imperative for this population.

This thesis demonstrates that there are noteworthy deficiencies in osteoporosis knowledge that need to be addressed using a multifaceted approach. It is worrisome that although the majority of the women claimed to have heard or read something about osteoporosis, over half

of them had not translated this information into higher scores on the FOOQ. Further studies are required to determine the level of knowledge and preventative behaviors among more heterogeneous samples of women. Our results also indicate that the role of exercise in the prevention of osteoporosis may be misunderstood by women in this age group. More studies are needed to examine the most beneficial intensity, duration, frequency and type of strength training exercise for improving bone health in postmenopausal women. Finally, it must be emphasized that although knowledge is requisite for health promotion and disease management, knowledge alone is not sufficient to promote behavior change. Even women who know that they are at risk and are fully aware of preventative strategies may not put those strategies into action. Nonetheless, understanding deficiencies in women's knowledge of osteoporosis is the first step toward developing preventative interventions.

Physician education has played a dominant role in strategies for raising awareness of osteoporosis and initiating appropriate treatments. The onus is on physicians to recognize the risk factors for osteoporosis and to educate, counsel, and treat their patients appropriately. In spite of this, in a study examining physicians' understanding of osteoporosis, Werner and Vered found that as many as 38% of the physicians surveyed underestimated the prevalence of the disease.(Werner & Vered, 2002) A recent study revealed that there are deficiencies in osteoporosis knowledge among nurses and other health professionals working with individuals who are at risk of osteoporotic fracture or have had a fracture.(Giangregorio *et al.*, 2007) In the study by Giangregorio *et al.*, a number of health professionals requested patient-related resources, including educational information on osteoporosis for patients on osteoporosis, and more time per patient, suggesting that they would like to play a larger role

in assisting with patient education.(Giangregorio *et al.,* 2007) It is noteworthy that in the present study, the family physician was identified most frequently as a source of information on osteoporosis. Other health professionals, that were cited included research and BMD technicians, but not nurses. This is inopportune, considering that nurses often have much more contact with patients at risk for the disease and could provide an educational asset by offering information regarding osteoporosis, its consequences, prevention, and treatment.(Ribeiro & Blakeley, 1997) The specific areas where women need information about osteoporosis concern lifestyle factors – such as the need for calcium in young and elderly women, the changes in bone due to menopause, the importance of weight-bearing exercise, and the implications of alcohol use. These are areas in which nurses, and other health care professionals, as well as media sources can make positive inroads through health education. Furthermore, health care professionals cannot assume that postmenopausal women have previously seen a health care provider who has offered them adequate information about osteoporosis risk factors and preventative behavior. Educating patients and healthcare providers about osteoporosis may play a pivotal role in improving osteoporosis management, which is the primary means of offsetting the occurrence of initial fragility fractures. Patients should be further encouraged to use their knowledge proactively to request more information about the disease and a thorough explanation of treatment alternatives from their physicians. An understanding of the characteristics of women with poor knowledge of osteoporosis may help researchers design more appropriate public health education programs.

DXA measurements of BMD are the accepted standard for diagnosing osteoporosis. This provides an estimate of the relative risk of fracture but its utility in individual patient risk

assessment is limited. Current evidence also suggests that BMD measurements may not have a dominant role in predicting clinical response to osteoporosis treatment. The limitations of BMD measurements in these clinical roles have prompted investigation for more reliable and responsive tools for measuring bone changes, such as pQCT technology. The advantage of detecting changes in specific bone compartments via pQCT are clearly evidenced in this thesis, where the longitudinal changes in cortical bone density were demonstrated at the radius and tibia in the breast cancer subjects with no significant change in total BMD. However, the interrelationships between skeletal sites and the clinical benefits of separate cortical and trabecular bone analysis, need to be more fully determined before they can be used directly in assessing fracture risk in the general population. It is foreseeable that osteoporosis will present a significant challenge to an aging population of breast cancer survivors, unless effective early detection and intervention strategies are developed and implemented. Despite increasing concerns regarding bone health in cancer survivors, data on BMD and rate of changes in BMD values among breast cancer survivors compared with non-cancer reference groups are still scarce. The results shown here have demonstrated the need for further research in this area. Future studies are necessary in order to investigate causes of bone loss in breast cancer survivors who have been diagnosed with cancer at different stages. Moreover, it is not yet clear how to interpret substantially different bone density, such as how the risk of certain types of fracture differs for women with high bone mass in the spine and low bone mass in the radius. These questions, along with a definitive framework for the interpretation of cortical and trabecular bone measurements, can only be resolved through continued investigation in this area.

Chapter 6

CONCLUSIONS

6.1 CONCLUSIONS

The objectives of the first study were to determine the mean values and the reproducibility of *in vivo* total, trabecular, and cortical volumetric bone measurements and muscle cross-sectional area in healthy pre- and postmenopausal women using the XCT-2000 pQCT scanner. Twenty-nine women (14 premenopausal and 15 postmenopausal) were recruited to participate in this study. Distal and diaphyseal sites of the radius (4% and 20% of the length of the radius) and tibia (4%, 38%, and 66% of the length of the tibia) were examined. At the distal tibia, the mean values for total ($p<0.05$) and trabecular ($p<0.01$) density were significantly lower in postmenopausal women than in premenopausal women. There were no significant differences detected at the distal radius, diaphyseal radius, or diaphyseal tibia. Of all sites, total density between the distal radius and diaphyseal radius correlated the strongest ($p=0.01$). The *rms*CV for the distal tibia gave the most favorable reproducibility values for total (1.5%) and trabecular (1.6%) density, while the diaphyseal tibia provided the most favorable value for cortical density (0.3%). The *rms*CV for muscle and subcutaneous fat cross-sectional area at the calf were 0.6% and 0.7% respectively. Overall, significant differences in volumetric bone measurements between healthy pre- and postmenopausal women were only evident at the distal tibia. Furthermore, the data presented here indicate that XCT-2000 scans at the tibia provide highly reproducible measurements of total, cortical, and trabecular bone as well as muscle and fat area.

Patient knowledge regarding osteoporosis is crucial to the implementation of preventative measures towards reducing the incidence of fragility fractures; yet, the level of patient knowledge is rarely assessed in health-care programs. For the effective management of

osteoporosis, patients must be aware of the various risk factors for this disease, as well as potential treatment options. The aim of the second study was to assess the level of osteoporosis knowledge in a population of postmenopausal women in order to compare results between women with and without a history of breast carcinoma. Fifty-eight women were recruited to participate in this study. Of this sample, twenty-seven subjects had a recent history of breast cancer diagnosis with ongoing treatment. All study participants completed the Facts on Osteoporosis Quiz (FOOQ) along with a medical history questionnaire. The mean score on the FOOQ for the healthy postmenopausal women (75%) was significantly higher ($p<0.01$) than the mean score achieved by the breast cancer patients (65%). Overall, the level of osteoporosis knowledge is considered to be low for both sub-groups, particularly in the areas of potentially preventative behavior. These areas of knowledge deficiency included the effects of alcohol consumption, weight-bearing exercise, calcium requirements, and early menopause on bone health. More patient education is needed regarding osteoporosis risk factors, potential preventative actions and treatment measures in postmenopausal women. In particular, women who are at increased risk for the development of osteopenia or osteoporosis due to their medical history, including women with a history of breast carcinoma, require a specialized strategy for the educational translation of osteoporosis knowledge as a principle aspect of their treatment.

pQCT scanners allow for the assessment of volumetric bone mineral density and the separate evaluation of cortical and trabecular bone compartments at peripheral skeletal sites. The primary objective of this thesis was to utilize pQCT to describe trabecular and cortical bone at the radius and tibia in postmenopausal women and to compare these values with those from

women with a history of breast carcinoma. Of particular interest was the effect of Anastrozole on the cortical and trabecular compartments of the radius and tibia. Fifty-eight women were recruited to participate in this study. Of this sample, 27 had a recent history of breast cancer diagnosis with ongoing treatment, and 31 were healthy postmenopausal women without underlying bone-related co-morbidities. The following measurement sites were significantly lower in the breast cancer subject group: TOT_DEN and TOT_CNT at the 4% radius; CRT_DEN, TOT_CNT, and CRT_CNT at the 20% radius; TOT_DEN at the 4% tibia; and CRT_DEN at the 38% tibia. With respect to time on Anastrozole, TOT_CNT at the 4% radius (r=-0.36); TOT_CNT (r=-0.33), CRT_CNT (r=-0.34) and CRT_DEN (r=-0.44) at the 20% radius; and CRT_DEN (r=-0.39) and CRT_CNT (r=-0.27) at the 38% tibia; were significantly negatively correlated with days on Anastrozole. In a small cohort of eight breast cancer subjects, longitudinal changes in trabecular and cortical volumetric bone density at the non-weight-bearing radius and weight-bearing tibia were assessed. Utilizing ANOVA, a significant decrease in CRT_DEN (p=0.025) was found at the 20% diaphyseal radius during the 2 year follow-up. At the 38% diaphyseal tibia, the decrease in CRT_DEN closely approached significance at p=0.051. In summary, this novel study reports significant side-to-side differences in volumetric bone density and content, particularly cortical density as measured by pQCT. It appears that time on Anastrozole primarily affects cortical bone density in these women. This study indicates a need for further prospective research and strongly suggests that additional research with a larger sample size of postmenopausal breast cancer subjects is warranted in order to evaluate the changes in trabecular and cortical bone, as well as to examine the incidence of fracture in response to Anastrozole therapy.

By way of conclusion, this thesis points to a deficit in knowledge concerning osteoporosis among postmenopausal women, particularly those with a history of breast carcinoma, while also demonstrating that these same subjects have an increased need to understand the preventative and treatment options regarding this disease, as they showed reduced total bone density at all four measurement sites. The results of this thesis call for a qualitative evaluation of public education and other health promotion efforts designed to improve women's knowledge and practices regarding osteoporosis, markedly for breast cancer survivors. Health-care professionals must be well prepared to educate the public, finding innovative and effective approaches for stressing the benefits of preventative actions. Practitioners need to provide individually suited information about osteoporosis to their individual patients, this includes: prescribing appropriate types and amounts of physical activity; discussing the importance of self-monitoring menstrual cycles, and the consequences of abnormal cycles and fluctuating estrogen levels as a woman ages; and relaying dietary factors besides calcium and vitamin D that affect risk, such as the effects of alcohol and caffeine on bone health. Breast cancer patients, due to their focus on cancer-related treatments, may fail to appreciate the long-term implications of osteoporosis and, in turn, may be less likely to absorb, retain and act on pertinent information about prevention and treatment. Furthermore, an underestimation of the severity of osteoporosis may contribute to low compliance rates among women who do initiate therapy.

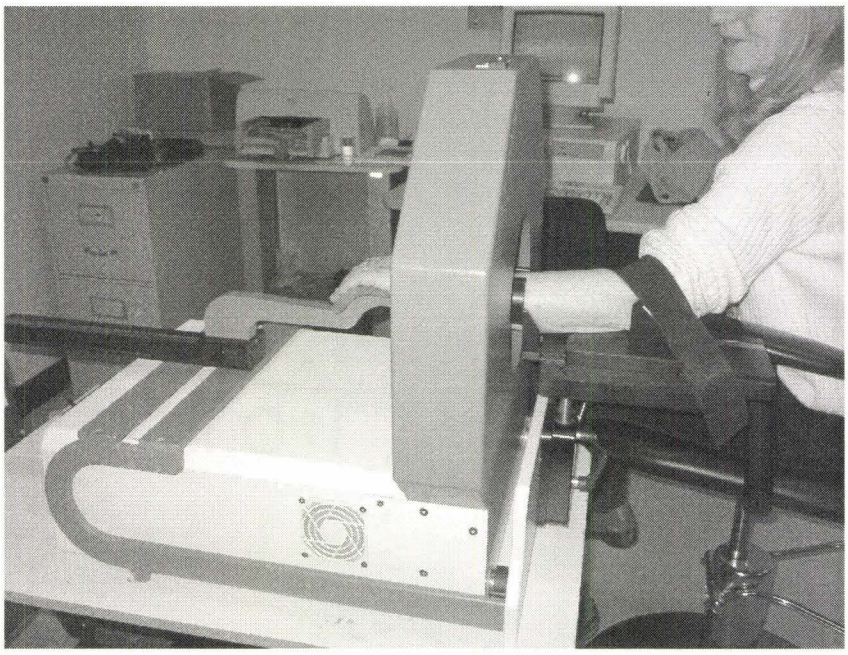
This thesis provides novel details regarding the differences in cortical bone in breast cancer subjects and emphasizes the need for a normative database of bone quality parameters at different skeletal sites in order to gain a better understanding of the utility of each skeletal site

with regard to fracture risk prediction. A possible approach to countering the rise of osteoporosis in breast cancer survivors is to develop an educational program tailored to the medical history and ongoing concerns of this specific population. Efforts to decrease the incidence of osteoporosis should include population-based intervention strategies targeted at alleviating the risk factors for osteoporosis, encouraging preventative self-care measures, and educating women about the importance of bone health. The development, evaluation, and dissemination of interventions designed to enhance knowledge of osteoporosis and encourage health-related behaviors will play a vital role in halting the progression of this silent epidemic.

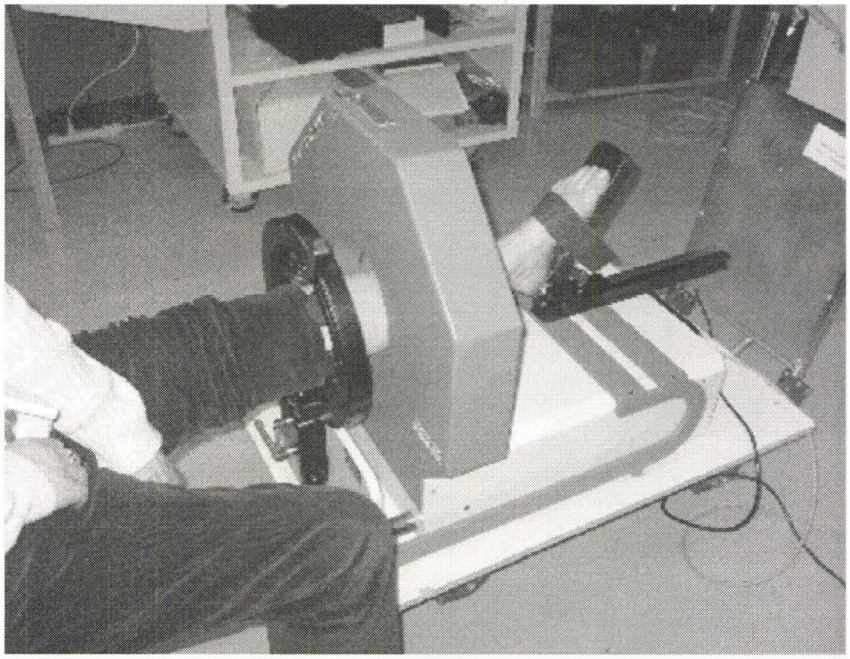
Appendices

APPENDIX A: POSITIONING OF THE PQCT DEVICE AT (A) THE RADIUS AND (B) THE TIBIA

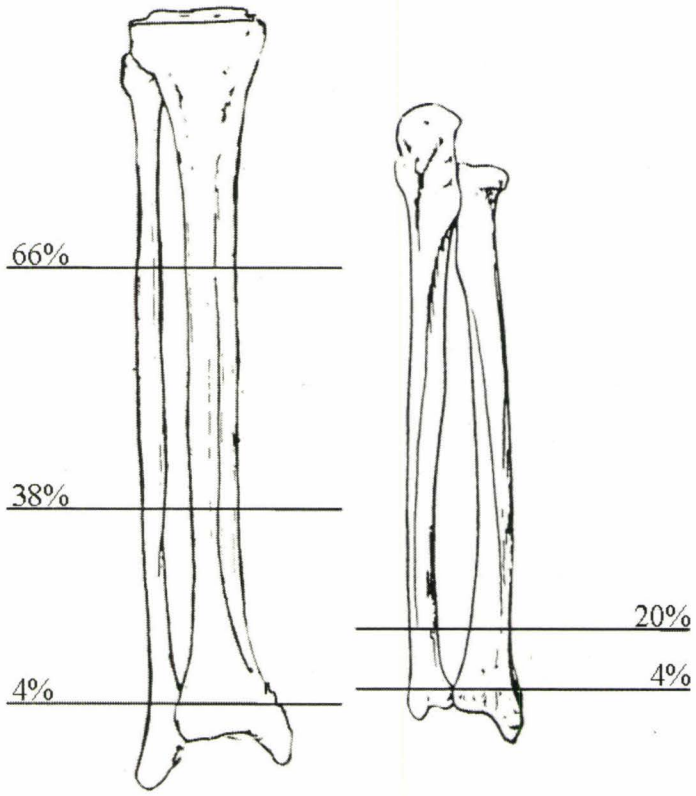
(A)



(B)

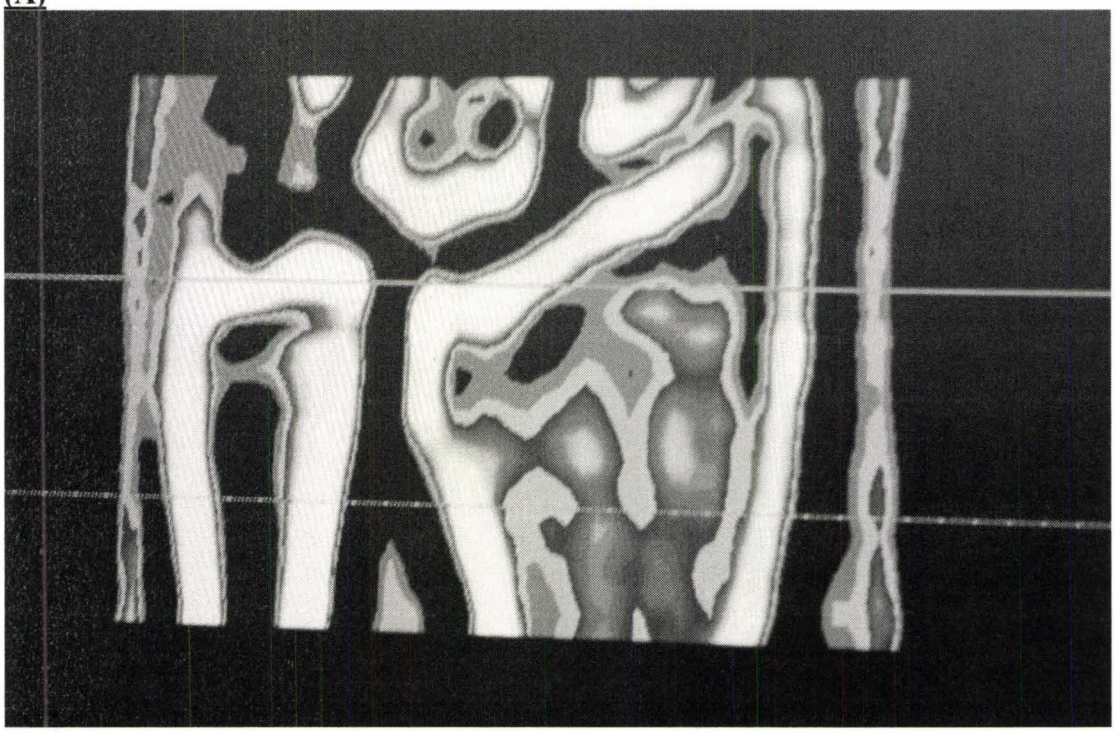


APPENDIX B: THE PQCT MEASUREMENT SITES OF THE TIBIA AND THE RADIUS

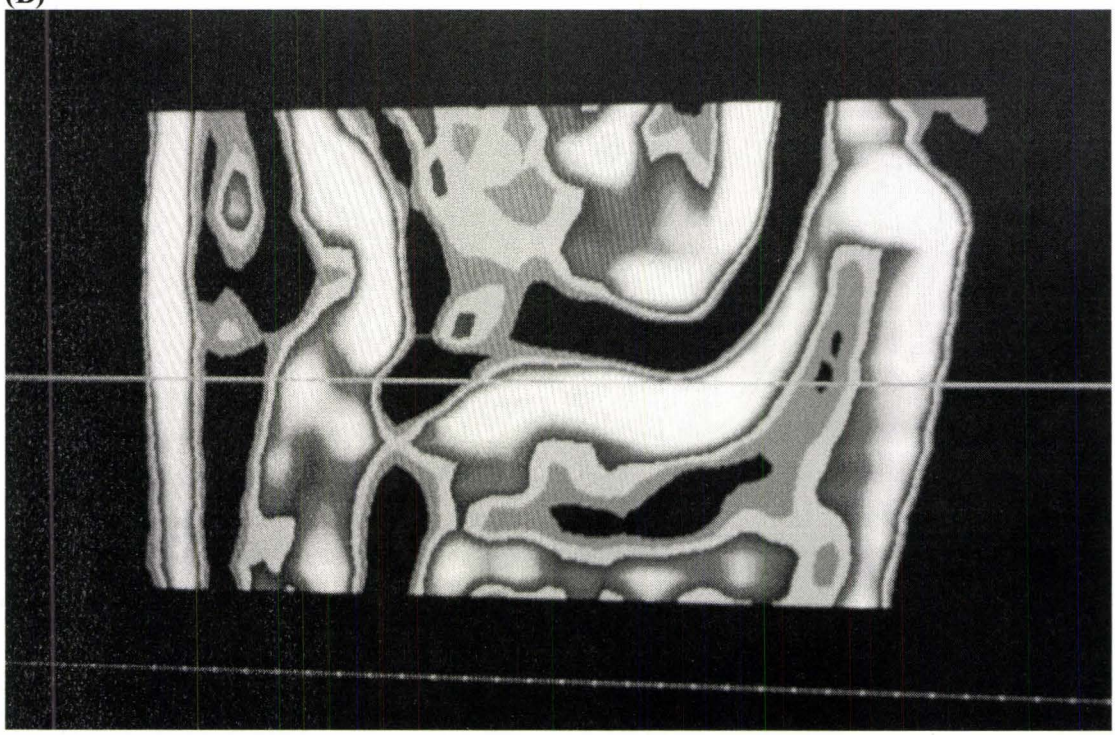


APPENDIX C: REPRESENTATIVE pQCT SCOUT SCANS OF (A) THE RADIUS AND (B) THE TIBIA

(A)

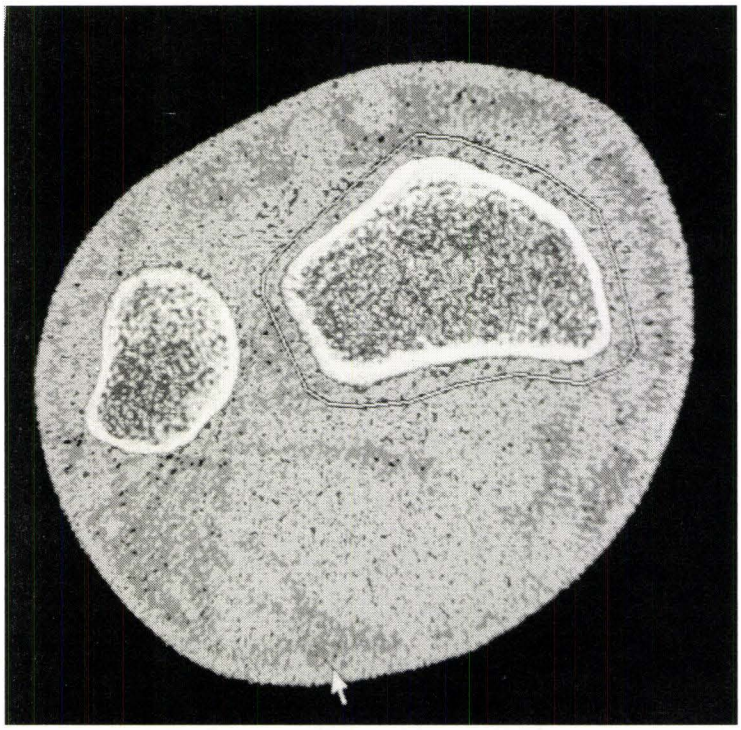


(B)

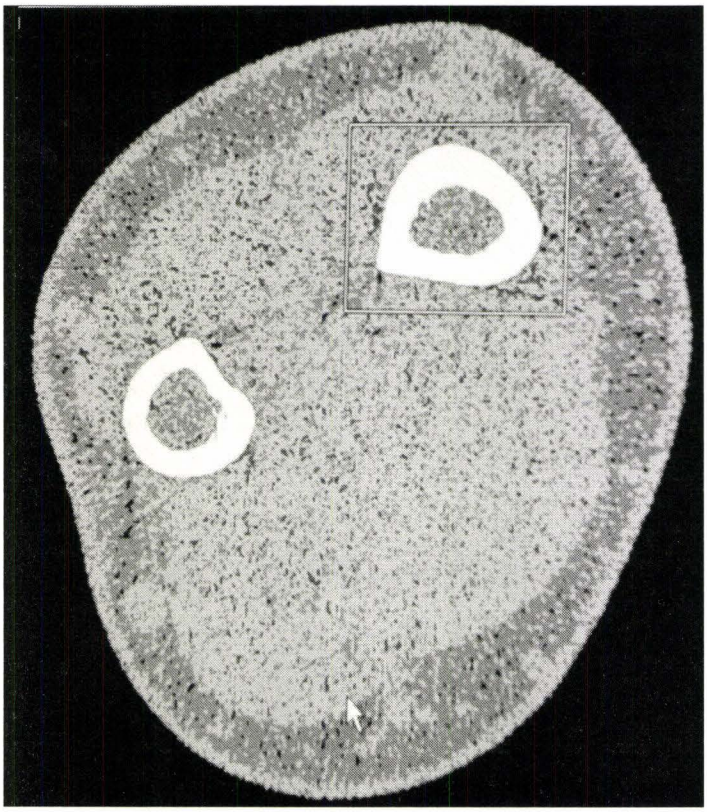


APPENDIX D: REPRESENTATIVE PQCT SCANS OF (A) THE 4% RADIUS AND (B) THE 20% RADIUS

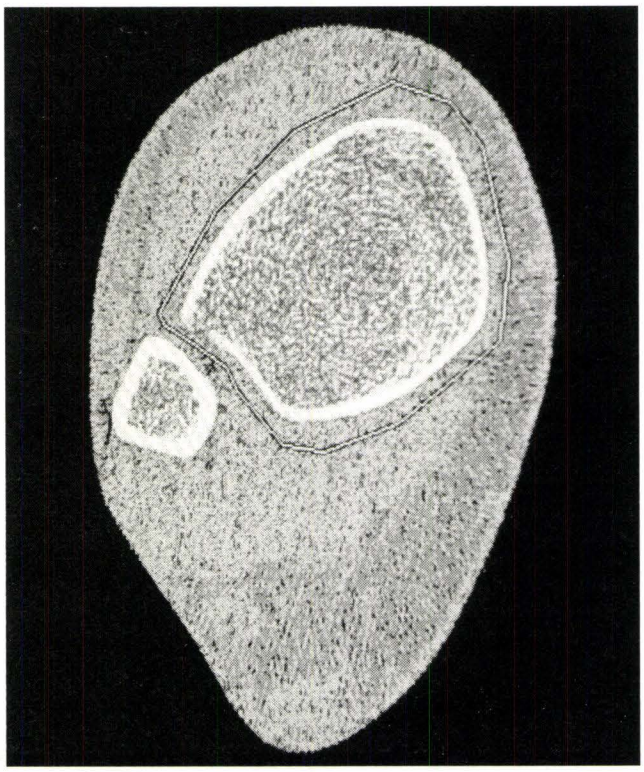
(A)



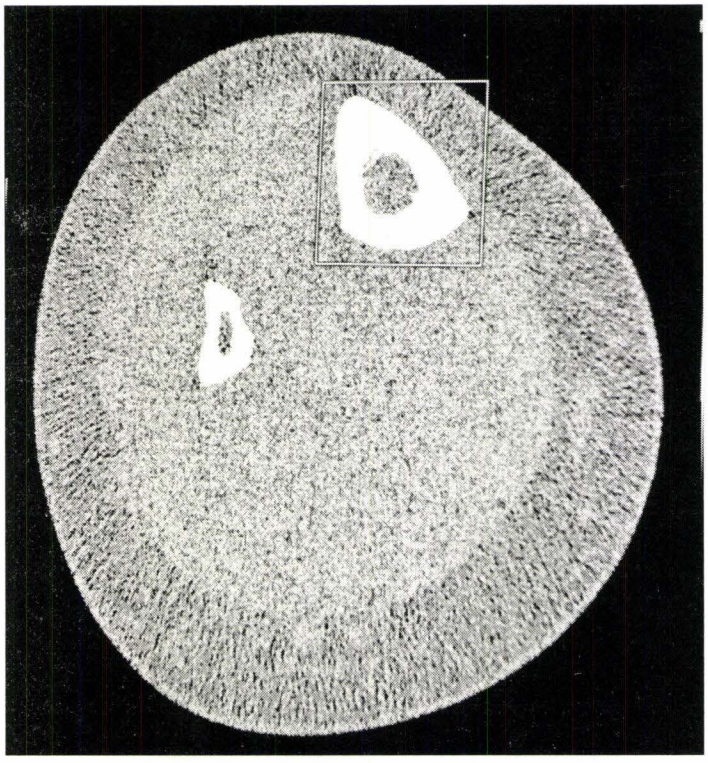
(B)



APPENDIX E: REPRESENTATIVE PQCT SCANS OF (A) THE 4% TIBIA AND (B) THE 38% TIBIA

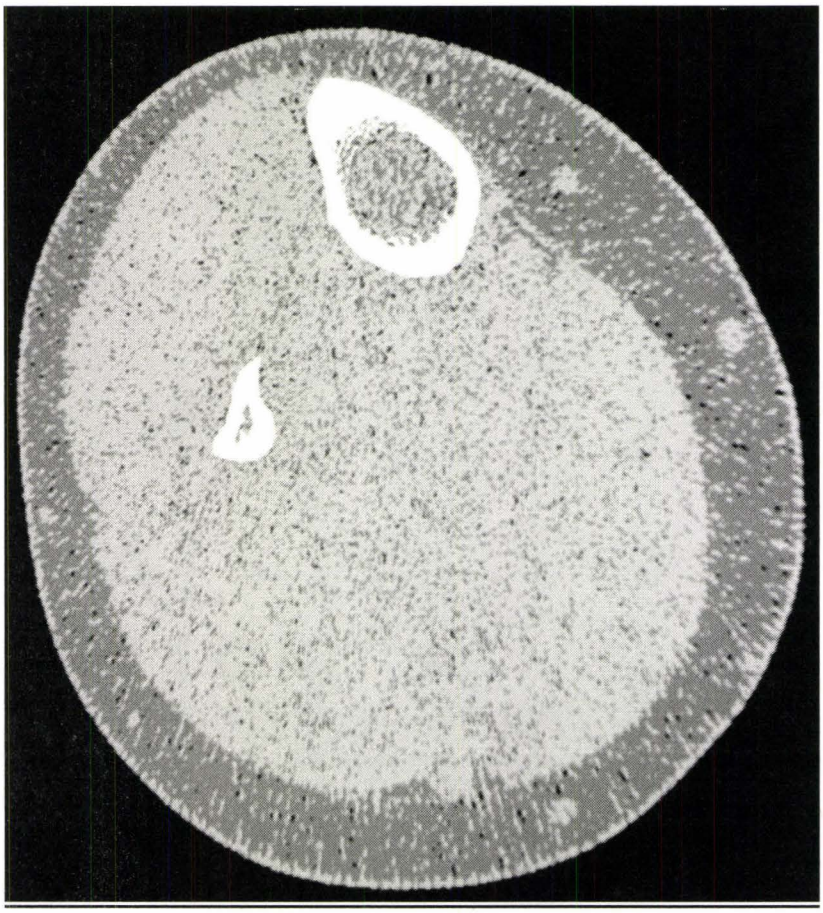


(A)



(B)

APPENDIX F: REPRESENTATIVE PQCT SCAN OF THE 66% TIBIA



APPENDIX G: FACTS ON OSTEOPOROSIS QUIZ - QUESTIONNAIRE PERMISSION LETTER

From: Margaret E Brierton
<meb76@georgetown.edu>

Subject: QUIZ

Date: Wed, 17 Jan 2007 09:46:32 -0500

To: szabok2@mcmaster.ca

Thank you for your interest in the Facts on Osteoporosis Quiz. I have attached to this message the quiz and the answers. Subject to the restraints specified below, you have permission to use the quiz in your research.

The copyright must appear on the printed copies of the instrument. The instrument will, of course, need to be appropriately reference in any published work. At this time there is no charge for the tool. In return for using the quiz, we ask that you send us copies of any publications citing the use of the quiz.

Please let me know by return email if you agree to these conditions and decide to use the FOOQ instrument. Please send me your complete address.

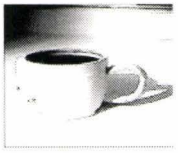
Sincerely,

Rita L. Ailinger (signed electronically)

Rita L. Ailinger, PhD, RN
Professor

APPENDIX H: FACTS ON OSTEOPOROSIS QUIZ



Facts On Osteoporosis Quiz

	Osteoporosis refers to weakened bone strength. It is commonly called "brittle bones" because this disease increases the risk of bone fractures. Completely fill in the circle of the appropriate answer.	True	False	Don't Know
1	Physical activity increases the risk of osteoporosis.	○	○	○
2	High impact exercise (weight training) improves bone health.	○	○	○
3	Most people gain bone mass after 30 years of age.	○	○	○
4	Low weight women have osteoporosis more than heavy women.	○	○	○
5	Alcoholism is not linked to the occurrence of osteoporosis.	○	○	○
6	The most important time to build bone strength is between 9 and 17 years of age.	○	○	○
7	Normally, bone loss speeds up after menopause.	○	○	○
8	High caffeine combined with low calcium intake increases the risk of osteoporosis.	○	○	○
9	There are many ways to prevent osteoporosis.	○	○	○
10	Without preventive measures 20% of women older than 50 years will have a fracture due to osteoporosis in their lifetime.	○	○	○
11	There are treatments for osteoporosis after it develops.	○	○	○
12	A lifetime of low intake of calcium and vitamin D does not increase the risk of osteoporosis.	○	○	○
13	Smoking does not increase the risk of osteoporosis.	○	○	○
14	Walking has a great effect on bone health.	○	○	○
15	After menopause, women not on estrogen need about 1500 mg of calcium (for example, 5 glasses of milk) daily.	○	○	○
16	Osteoporosis affects men and women.	○	○	○
17	Early menopause is not a risk factor for osteoporosis.	○	○	○
18	Replacing hormones after menopause cannot slow down bone loss.	○	○	○
19	Children 9 to 17 years of age get enough calcium from one glass of milk each day to prevent osteoporosis.	○	○	○
20	Family history of osteoporosis is not a risk factor for osteoporosis.	○	○	○

APPENDIX I: FACTS ON OSTEOPOROSIS QUIZ - ANSWER KEY PROVIDED BY THE AUTHORS

Facts on Osteoporosis Quiz

Osteoporosis refers to a loss of bone mass.

1. *Physical activity increases the risk of osteoporosis.* **FALSE**
   Physical activity is necessary to build and maintain bones throughout life. Inactivity increases the risk of osteoporosis.

2. *High impact exercise (weight training) improves bone health.* **TRUE**
   High-impact exercise (especially resistance activities such as weight training) stimulates accumulation of bone mineral content in the skeleton and leads to peak bone mass. Additionally, high- impact exercises may decrease the risk of falls in older adults.

3. *Most people gain bone mass after 30 years of age.* **FALSE**
   Bone loss commonly occurs as men and women age. Men do not lose bone as soon or as rapidly as women and experience only half the age-related bone loss of women. For women, the rapid loss of bone density is linked to declining estrogen levels associated with menopause.

4. *Low weight women have osteoporosis more than heavy women.* **TRUE**
   Low weight women are at higher risk than heavy women for osteoporosis.

5. *Alcoholism is not linked to the occurrence of osteoporosis.* **FALSE**
   Alcohol abuse is associated with osteoporosis and increased fracture risk. The diets of those who abuse alcohol can contain too little calcium, vitamin D, and other nutrients necessary to maintain bone mass.

6. *The most important time to build bone strength is between 9 and 17 years of age.* **TRUE**
   Growth in bone size and strength occurs during childhood although bone accumulation is not completed until about age 30. An individual who does not reach optimal bone mass during childhood and adolescence may develop osteoporosis without the occurrence of accelerated bone loss.

7. *Normally, bone loss speeds up after menopause.* **TRUE**
   During the first three years after menopause, accelerated bone loss occurs in one of every four women. These women may lose as much as 15% of bone mass each year for a total of 45% during a three-year period.

8. *High caffeine combined with low calcium intake increase the risk of osteoporosis.* **TRUE**
   High caffeine intake can adversely affect calcium balance in individuals with an inadequate intake of calcium.

9. *There are many ways to prevent osteoporosis.* **TRUE**
   Efforts to optimize bone mass, such as exercise, good nutrition and appropriate intake of calcium and vitamin D, in the first 30 years of life help to prevent osteoporosis. Risk factors, such as smoking and alcohol abuse, can be corrected.

10. *Without preventive measures, 20% of women older than 50 years will have a fracture due to osteoporosis in their lifetime.* **TRUE**
    The probability that a 50-year old will have a hip fracture during their lifetime is 14% for a white females and 6% for African American females.

11. *There are treatments for osteoporosis after it develops.* **TRUE**

In the past 30 years, great strides have been made in the treatment of osteoporosis. However, no therapy is totally effective in reversing the process of osteoporosis.

12. *A lifetime of low intake of calcium and vitamin D does not increase the risk of osteoporosis.* **FALSE**
Research shows that lifetime intake of calcium and vitamin D are critical factors in osteoporosis. Calcium is the specific nutrient that is most important for attaining peak bone mass and vitamin D is required for optimal calcium absorption.

13. *Smoking does not increase the risk of osteoporosis.* **FALSE**
Cigarette smoking, which usually starts in adolescence, may have a deleterious effect on achieving bone mass.

14. *Walking has a great effect on bone health.* **FALSE**
Low impact exercise, such as walking, has beneficial effects on other aspects of health and function. However, the effects of low-impact exercise on bone mineral density have been minimal.

15. *After menopause, women not on estrogen need about 1,500 mg of calcium (for example, 5 glasses of milk) daily.* **TRUE**
Women not on estrogen after menopause should have a calcium intake of 1,500 mg daily, the amount of calcium contained in 5 glasses (8 ounces) of milk.

16. *Osteoporosis affects men and women.* **TRUE**
Osteoporosis affects approximately 10 million Americans: of these, 2 million are men.

17. *Early menopause is not a risk factor for osteoporosis.* **FALSE**
In several studies, early menopause has been found to be associated with low bone mineral density which is associated with increased risk of osteoporosis.

18. *Replacing hormones after menopause cannot slow down bone loss.* **FALSE**
After menopause, hormone replacement therapy can slow down bone loss.

19. *Children 9 to 17 years of age get enough calcium from one glass of milk each day to prevent osteoporosis.* **FALSE**
An 8 ounce glass of milk contains only 300 mg of calcium. Children 9 to 17 years of age would have to drink four to five 8 oz. glasses of milk each day to get the recommended 1,300 mg of calcium per day.

20. *Family history of osteoporosis is not a risk factor for osteoporosis.* **FALSE**
Genetic factors such as family history exert a strong influence on peak bone mass but physiological, environmental, and lifestyle factors that can be modified also play a significant role in osteoporosis.

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