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PQCT MEASUREMENTS OF BONE MASS, DENSITY, GEOMETRY AND

STRUCTURE AT THE DISTAL RADIUS:

PREDICTION OF BONE STRENGTH, AND THE *IN VIVO* EFFECTS OF

PHARMACOLOGICAL TREATMENTS FOR OSTEOPOROSIS

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

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THE RADIUS: BONE STRENGTH AND *IN VIVO* RESPONSE TO MEDICATIONS

DOCTOR OF PHILOSOPHY (2003)   McMaster University
(Medical Sciences)   Hamilton, Ontario

TITLE: PQCT Measurements of Bone Mass, Density, Geometry and

                    Structure at the Distal Radius: Estimation of Bone Strength,

                    and the *In Vivo* Effects of Pharmacological Treatments for

                    Osteoporosis.

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NUMBER OF PAGES: *x*, 245

ABSTRACT

This work encompasses two primary studies utilizing peripheral quantitative computed tomography (pQCT) in the assessment of bone characteristics at the distal radius. PQCT enables separate analyses of cortical, trabecular and total bone density, content and geometry. Further analysis of the pQCT image with specialized software permits *in vivo* trabecular structure assessment.

The first study is a two-year prospective trial evaluating the effects of five different medication regimens (hormone replacement therapy (HRT), etidronate , alendronate, etidronate plus HRT and alendronate plus HRT) used for the treatment of osteoporosis in 123 postmenopausal women with low bone mass. Results of analyses on baseline data found that trabecular structure indices could significantly discriminate between osteoporotic and osteopenic groups of women. Longitudinal data at the lumbar spine (LS) and femoral neck (FN) for women taking any medication ('Any-Tx') showed similar results to those of large clinical trials. For example, the 'Any-Tx' group gained significant bone mineral density (BMD) over baseline at 2 years at the LS (5.5%, $p < 0.001$) and FN (1.6%, $p < 0.05$). Women on combination therapy gained significantly more BMD than women taking one medication over 2 years at the LS and FN. Data suggests that alendronate plus HRT may produce the largest BMD gains at the LS and FN at 2 years.

Longitudinal pQCT data for total, cortical and trabecular compartments at the distal radius in the control group over the 2 years suggest endocortical resorption and trabecular

thinning with age. PQCT data in the 'Any-Tx' group show gains in total and cortical bone density and cortical content, but losses in trabecular density, content and area. This suggests that anti-resorptive medications promote endocoritcal apposition and reduce intracortical porosity.

The second study evaluated five different clinical measurement tools in the prediction of *in vitro* failure load at the distal radius and found that cortical content measured by pQCT was a significantly better predictor than ultrasound or digital x-ray radiogrammetry.

ACKNOWLEDGEMENTS

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LIST OF ACRONYMS

Bone measurement techniques			
DXA	dual energy x-ray absorptiometry	pQCT	peripheral quantitative computed tomography
DPA	dual photon absorptiometry	DXR	digital x-ray radiogrammtery
SXA	single energy x-ray absorptiometry	Accu-DXA	DXA of the phalanges (finger bones)
SPA	single photon absorptiometry	QUS	quantitative ultrasound

Bone measurement at the hip and spine by DXA			
BMD	bone mineral density	BMC	bone mineral content
LS	lumbar spine	FN	femoral neck

Bone measurement at the radius by pQCT			
CSA	cross-sectional area	TOT	total
vBD	volumetric bone density	TRAB	trabecular
CNT	bone content	CRT	cortical
Ha	average(mean) hole size		
Hm	maximum hole size	Ix	moment of inertia
CI	connectivity index	SSI-p	polar stress-strain index

Pharmocological Treatment Regimens			
ALN	alendronate	Any-Tx	Any of the 5 treatments (HRT, ALN, ETD, A+H, E+H)
ETD	etidronate		
HRT	hormone replacement therapy	1-Tx	Any of the single therapies (HRT, ALN or ETD)
E+H	etidronate plus HRT		
A+H	alendronate plus HRT	2-Tx	Any of the combination therapies (A+H or E+H)
CNTL	control (no therapy)		

CHAPTER 1: INTRODUCTION

1.1 Osteoporosis

1.1.1 Definition

Osteoporosis is defined as 'a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture' especially at the hip, spine and the distal radius[Consensus Development Conference 2000]. The current diagnostic criteria for Caucasian women depend on the measurement of bone mineral density (BMD) and comparison of a patient's BMD to the young adult peak BMD. This comparison is quantified in standard deviations and yields a T-score. If a patient's BMD is 2.5 standard deviations or more below the young normal value (T-score $\leq$ -2.5), then they are considered to have osteoporosis. The presence of a fracture accompanying a T-score $\leq$ -2.5 would place the patient into a classification of severe osteoporosis. Patients with T-scores between -1 and -2.5 are classified as having low bone mass or osteopenia and considered at risk for osteoporosis and therefore may benefit from preventative therapy[Kanis et al. 1997].

1.1.2 Prevalence and Projections

In 1993 in Canada there were an estimated 1.8 million women with osteoporosis and 60,000 fractures occurred[Goeree et al. 1996]. The treatment of these fractures each

year costs around $1.3 billion[Goeree et al. 1996]. Osteoporosis is generally thought of as a disease of the elderly since it usually affects women after 50 years of age and men even later. With the proportion of the elderly in our population increasing, projections suggest that the number of hip fractures in Canada will rise exponentially over the next 40 years, from approximately 23,000 hip fractures per year in 1993/1994 to over 88,000 in 2041[Papadimitropoulos et al. 1997]. In 1990 there were an estimated 1.26 million hip fractures worldwide. Estimates predict 4.5-37.2 million hip fractures worldwide by 2050 depending on age- and gender-specific trends[Gullberg, Johnell, and Kanis 1997]. Clearly, it is important to manage this disease and attenuate its effects on society.

1.1.3 Fracture

The detrimental health effects of osteoporosis and its monetary burden are due to fracture and not specifically the disease (low bone mass) itself. The disease is simply an indicator or risk factor for an increased susceptibility to fracture. Fracture occurs when the force on a bone exceeds the strength of that bone[Bouxsein, Myers, and Hayes 1996]. Most fractures in osteoporotic patients occur with little force, since their bones are weakened, and thus are termed fragility fractures. The rate of osteoporotic fractures is higher in women than men and generally increases with age[Melton 1995]. It is these type of fractures that osteoporosis treatment aims to reduce.

1.1.3.1 Determinants of Fracture

There are two main determinants of fracture: the force acting on the bone (non-skeletal factors) and the strength of the bone itself (skeletal factor). Some of the non-skeletal factors include visual impairment, neuromuscular impairment and body habitus [Cummings et al. 1995; Dargent-Molina et al. 1996]. These non-skeletal factors will affect the frequency, severity and direction of the trauma or fall. Non-skeletal factors are beyond the scope of this thesis. Bone strength is discussed in more detail in Chapter 2.

1.1.3.2 Location of Osteoporotic Fractures

The majority of osteoporotic fractures occur at three sites: the spine, hip and radius. Other less common sites of osteoporotic fracture include the proximal humerus, ribs, pelvis, distal femur and proximal and distal tibia[Melton 1995]. Vertebral fragility fractures are the most common osteoporotic fractures, but are sometimes difficult to diagnose since many occur with the absence of pain or symptoms. Patients with spinal crush fractures permanently lose height and with the accumulation of a number of wedge-type fractures, can develop marked kyphosis[Dempster and Lindsay 1993]. This concave-anterior curvature of the thoracic spine compresses the abdominal contents and can disrupt digestion as well as hinder regular respiration since the diaphragm has difficulty descending with contraction. Kyphotic patients also have trouble raising their head to look forward and so must turn their head to the side in order to peer upwards. Extreme cases can eventually lead to the lower ribs of the patient resting on the superior aspect of

the pelvis (iliac crest). Patients suffering from vertebral fragility fractures may also have to contend with chronic back pain[Dempster and Lindsay 1993]

Proximal femur (hip) fractures usually require surgery. Some patients may be able to successfully rehabilitate to their pre-injury functional level, but many are likely to experience a decrease in mobility and activity level[Craik 1994]. A more serious complication of hip fracture is the increased mortality. The number of patients who will not survive the first year following the injury is high at 17-33%[Keene, Parker, and Pryor 1993; Forsen et al. 1999]. Hip fractures are also responsible for the majority of the economic burden due to the treatment of osteoporosis[Goeree et al. 1996].

Wrist fragility fractures, which occur at the distal radius, are also known as Colles' fractures. These fractures usually occur during a fall onto an outstretched hand. In Colles' fracture there is swelling of the wrist and movement is usually limited by pain in the acute injury. Complications include deformity, stiffness of wrist, forearm and even shoulder, carpal tunnel syndrome and occasionally rupture of tendons of the fingers or the thumb[Wadsworth 1990].

1.1.4 Bone Changes with Age

1.1.4.1   Peak Bone Mass

Adult bone mass is determined by two variables: peak bone mass and rate of bone loss. The age at which peak bone mass is attained is somewhat under debate. What does seem to be agreed upon is that peak bone mass at the hip is reached earlier than peak bone

mass at the spine. The age that peak bone mass is attained ranges from 20-29 years of age for the hip and 20-39 years for the spine[Haapasalo et al. 1996; Diaz et al. 1997; Blanchet et al. 1998]. A large Spanish study of 2442 subjects showed bone density at the spine to peak one decade earlier in men than women (20-29 years in men versus 30-39 years in women)[Diaz et al. 1997]. This same study also found that bone density at the hip and spine was significantly higher in men than in women for all age groups except in the 20-39 year old group for the spine[Diaz et al. 1997].

1.1.4.2 Bone Loss with Age

Evidence for premenopausal and perimenopausal bone loss is controversial. Diaz et al. (1997) studied bone density in subjects from 20 to 80 years old and found that bone density in women remained stable until age 39 years at the spine and until 49 years at the hip[Diaz et al. 1997]. Other studies support the finding that bone density in women remains stable until menopause. Lofman et al. (1997) discovered no significant change in BMD at the hip, spine or forearm in women between 20 and 49 years of age, and Duboeuf et al.(2000) found no significant bone loss at the forearm in 138 premenopausal women over two years. Some studies have found small decreases in bone density at the spine and/or hip occurring before the menopause[Ravn et al. 1994; Blanchet et al. 1998]. In contrast, a recent 3-year longitudinal study in 272 women aged 31-59 years detected small but significant bone density increases at some sites in the hip, the radius and at the spine in premenopausal women[Chapurlat et al. 2000].

When women reach the age of around 49, and estrogen levels decline with menopause, there are substantial losses in BMD at the hip, spine and forearm[Lofman et al. 1997; Duboeuf et al. 2000]. Lofman et al.(1997) found that 75-88% of the bone loss between ages 30 and 69 years occurred in the ten years immediately following menopause. The finding of accelerated postmenopausal bone loss is supported by recent studies which have shown that in the 5 years immediately following menopause women lose bone at a rapid rate of 1-4% per year at the lumbar spine as their estrogen level and its' bone sparing effects diminish[Okano et al. 1998; Mazzuoli et al. 2000]. Due to this rapid loss of bone mass at around 50 years of age and a lower peak bone mass than men, older women tend to be affected by osteoporosis much more readily than men are. When hip BMD is used for diagnosis, osteoporosis was found to be 3-8 times more prevalent in women over 50 years old than men over 50[Looker et al. 1997].

1.2   The Distal Radius

1.2.1   Areal Bone Density Changes at the Distal Radius with Age in Women

Peak bone mass at the distal radius has been found to occur later than at the hip and spine. Generally, bone mass is gained up until the ages between 31-49 years, then bone mass starts to decrease immediately and rapidly when estrogen is lost at menopause[Haapasalo et al. 1996; Lofman, Larsson, and Toss 2000; Nakamura et al. 2000; Berntsen et al. 2001]. The rate of postmenopausal bone loss in the radius in cross-sectional and longitudinal studies has been reported at 1.3-2.5%/year at the distal forearm

using SPA, SXA and DXA[Lofman et al. 1997; Duboeuf et al. 2000; Nakamura et al. 2000; Berntsen et al. 2001] and 1.5%-2.7%/year at the ultradistal radius with SXA[Duboeuf et al. 2000; Berntsen et al. 2001]. Lofman et al.(1997) found that 75% of the bone lost at the forearm between the ages of 30-69 years occurred in the first 10 years immediately following menopause. This rapid bone loss after menopause helps to explain the rise in Colles' fracture incidence in women between 40 and 65 years of age[Cooper and Melton 1992].

1.2.2   Volumetric Bone Density Changes at the Distal Radius with Age in Women

Studies using SXA and DXA measurements of BMD at the distal radius in postmenopausal women have shown decreases in areal BMD with age, but these studies have evaluated integral or total bone, and have not evaluated the trabecular and cortical bone compartments separately[Lofman et al. 1997; Duboeuf et al. 2000; Nakamura et al. 2000; Berntsen et al. 2001]. Research employing pQCT technology has shown that total volumetric bone density remains relatively stable at the distal radius until menopause, and then shows similar yearly losses (0.6-2.3% loss/year) to areal bone density[Ruegsegger, Durand, and Dambacher 1991; Butz et al. 1994; Gatti et al. 1996; Schneider et al. 1999; MacIntyre, Adachi, and Webber 1999; Guglielmi et al. 2000]. When trabecular and cortical compartments are considered separately, however, some pQCT studies have suggested that bone in the trabecular and cortical compartments may act differently with age in postmenopausal women[Ruegsegger, Durand, and Dambacher 1991; Ruegsegger, Durand, and Dambacher 1991; Nijs et al. 1998; Martin, Campbell, and Reid 1999;

Tsurusaki, Ito, and Hayashi 2000]. Two longitudinal studies using a high-resolution

(0.2mm) Densiscan system have reported greater yearly losses for postmenopausal

women in the trabecular core than in a highly localized cortical region of interest that lies

entirely within the cortical shell[Ruegsegger, Durand, and Dambacher 1991A;

Ruegsegger, Durand, and Dambacher 1991B]. This type of cortical analysis reduces the

partial volume effect, which occurs in the voxels at the edge of the bone. Rates of loss in

these two studies were 2.8% and 1.2% per year in trabecular bone in early

postmenopausal (n=20 for both studies) and osteoporotic (≥1 vertebral fracture, n=20)

women, respectively. In contrast, cortical bone density losses were insignificant at 0.0-

0.2% per year[Ruegsegger, Durand, and Dambacher 1991A; Ruegsegger, Durand, and

Dambacher 1991B]. A 4-year study using the same scanning system found a similar

trabecular rate of loss (-2.3%/y), and slightly higher cortical losses (-1.2%/y) in 38

postmenopausal women, but the cortical rate of loss was still slower than that of the

trabecular core[Tsurusaki, Ito, and Hayashi 2000]. Supporting these findings, a cross-

sectional study in 275 postmenopausal women reported greater losses at the distal radius

in the trabecular (-1.1%/y) than cortical (-0.6%/y) compartments[Nijs et al. 1998]. Other

cross-sectional studies have found comparable rates of loss in the cortical and trabecular

compartments[Gatti et al. 1996; Boonen et al. 1997], while one cross-sectional study has

reported a slightly faster rate of loss in the cortical compartment than the trabecular

core[Martin, Campbell, and Reid 1999].

1.2.3   Choice of the Distal Radius as a Bone Measurement Site

1.2.3.1   Clinical Significance of the Distal Radius Site

Fractures of the distal radius are the most common fractures in women less than 75

years of age[Owen et al. 1982]. The incidence of Colles' fracture in women peaks at

around 65 years of age, well before the maximum incidence for spine and hip

fractures[Cooper, Campion, and Melton 1992]. Since these fractures occur approximately

10 to 15 years before hip and spine fractures, they may be important, early indicators of

future fracture risk. Indeed, several studies have shown that a positive history for Colles'

fracture is a predictor of future fractures[Gardsell et al. 1993; Mallmin et al. 1993;

Cuddihy et al. 1999]. Specifically, the presence of a Colles' fracture substantially

increases an individual's risk for both hip (1.4 fold in women, 2.7 fold in men) and

vertebral fractures (5.2 fold in women, 10.7 fold in men)[Cuddihy et al. 1999].

Furthermore, BMD of the forearm has been shown to be a moderate predictor of distal

radius, hip and spine fractures (relative risks (RR) = 1.7, 1.8 and 1.7, respectively and

95% Confidence Intervals (CI_{95}) = 1.4-2.0, 1.4-2.2 and 1.4-2.1, respectively)[Marshall,

Johnell, and Wedel 1996]. Taken together, these observations indicate that the distal

radius could play an important role in the early detection of individuals at risk for

osteoporotic fracture.

1.2.3.2   Anatomy of the Distal Radius Site

The anatomy of the distal radius affords many advantages when measuring bone characteristics at this site. Since the distal radius is a peripheral site, it is easy to locate, access and measure. The fact that there is little soft tissue surrounding the bones at the wrist helps to improve accuracy and precision[Augat, Fuerst, and Genant 1998]. Another advantage of the distal radius is that there is both cortical and trabecular bone at this site, which allows for assessment of each compartment separately. As already presented in section 1.2.2, studies employing pQCT technology have detected differences in the way that the cortical and trabecular compartments respond to aging in postmenopausal women. Finally, the effective dose of a radiation-based measurement of bone at the distal radius is reduced because it is a peripheral site[Kalender 1992].

1.3   Outline of Thesis Research

This thesis is composed of two main studies. The first is a clinical study to determine how different pharmacological treatments for osteoporosis affect bone characteristics at the distal radius. The second is a cadaver study to determine which bone characteristics of the radius are important with respect to bone strength, as measured by radial failure load.

Indirectly, through a linkage of these two studies, it may be possible to infer which treatments alter radial bone strength by their effects on the measured bone characteristics. The first step will be to determine which bone variables are the best predictors of radial failure load. The second step will be to determine which treatment regimens improve

those specific radial bone variables the most. This information together then suggests

which treatments improve radial bone strength.

1.3.1 Study 1 - Prospective Clinical Trial:

Effects of Pharmacological Treatments for Osteoporosis on the Distal Radius

The focus of the clinical study in my thesis is to determine how accepted

pharmacological treatments for osteoporosis affect the bone mass, geometry and

trabecular structure of the distal radius in a head-to-head prospective 2-year study

including a control group. As detailed in section 2.1.3.2 shifts in bone mass from one

bone compartment (cortical or trabecular) to another are not necessarily detected using

dual-energy x-ray absorptiometry (DXA), but can be detected with peripheral quantitative

computed tomography (pQCT). The clinical study in my thesis utilizes pQCT technology

in order to determine whether there are any discrete changes in trabecular and cortical

bone mass at the distal radius with treatment, which have not previously been detected

using DXA. As well, changes in trabecular structure at the distal radius are monitored

with specialized software, which analyzes the cross-sectional image acquired by pQCT, to

determine whether pharmacological treatments affect trabecular architecture.

The objective in this study is to evaluate the effects of different pharmacological

treatments for postmenopausal osteoporosis, alone or in combination, on the distal radius

as measured by pQCT. The three anti-resorptive medications included in this study are

investigated in three separate approaches. First they are examined as a collective

treatment group versus a non-treatment group. Secondly, the treatment regimens are

considered as subjects taking any single anti-resorptive medication versus subjects taking

two anti-resorptive drugs (a bisphosphonate and hormone replacement therapy) versus

subjects taking no medication. Lastly, these treatments are analyzed as the 6 individual

treatment regimens (see section 4.4.1 for details on cohort division into treatment groups).

The primary endpoint of this study is:

> Bone mass and geometry changes in the total, trabecular and cortical bone

   compartments at the distal radius with treatment, as measured by pQCT

Secondary endpoints include:

> Bone density changes at the hip and spine with treatment, as measured by DXA

   (to demonstrate consistency with previous large randomized controlled trials)

> Trabecular structure changes at the distal radius with treatment

1.3.2 Study 2 - **In Vitro** *Mechanical Loading Study:*

Clinically Measurable Determinants of Radial Bone Strength

The focus of the *in vitro* study of my thesis is to determine which clinically available

bone measurement, or combination of measurements, is the best predictor of bone

strength at the distal radius. Bone strength is measured as radial failure load in a

mechanical testing set-up designed to mimic a fall on an outstretched hand that would

produce a Colles' fracture. The modalities tested were DXA, pQCT, quantitative

ultrasound (QUS) and digital x-ray radiogrammetry (DXR) of the forearm and DXA of

the phalanges. These techniques measure many different bone characteristics including

bone mass and density (DXA, pQCT, DXR), geometry (pQCT), trabecular structure

(pQCT) and speed of sound (QUS) in the radius. This study is presented in its' entirety in Chapter 7, formatted for and accepted by the journal 'Osteoporosis International' for publication.

CHAPTER 2: BONE MEASUREMENT TECHNIQUES AND THEIR RELATION TO BONE STRENGTH

2.1 Bone Measurement Techniques

The ultimate goal of skeletal measurement is to determine bone strength. However, since bone strength can only be measured by material testing (breaking) of the bone *in vitro*, surrogate measures of bone strength must be used for *in vivo* evaluation. Many different technologies exist to characterize bone in an individual *in vivo*. Some of these technologies include single and dual photon absorptiometry (SPA and DPA), single and dual x-ray absorptiometry (SXA and DXA), radiographic absorptiometry (RA), digital x-ray radiogrammetry (DXR), quantitative computed tomography (QCT), and quantitative ultrasound (QUS) [Genant et al. 1996; Jorgensen et al. 2000].

2.1.1 Single Energy Absorptiometry

Single photon absorptiometry (SPA) and single x-ray absorptiometry (SXA) measure peripheral sites. The number of photons attenuated by the measurement site depends upon the amount of bone and soft tissue in the path of the photon beam. These scanners must use a constant thickness of a soft-tissue equivalent around the scanned area, such as a water bath to help distinguish attenuation by soft-tissue from attenuation by bone mineral. The soft tissue equivalent material acts as a baseline that is subtracted

from the combined bone and soft tissue measurement to give a bone-only value[Augat,

Fuerst, and Genant 1998].

2.1.2 Dual Energy Absorptiometry

In dual-energy scanners the addition of a second radiation source of different energy

has improved upon single energy technology and allows scanning without the use of a

soft tissue equivalent. The two energy levels, distinguishing soft tissue from bone, also

allow for scanning of proximal or axial sites that contain a variable thickness of

surrounding soft tissue such as the spine or proximal femur[Genant et al. 1996]. Dual

energy systems, with the ability to directly measure axial and peripheral sites involved in

osteoporotic fracture, have mostly replaced single energy systems. Dual photon

absorptiometry, using a radionuclide source, was the first of the dual energy technologies

to be developed. Today, increased resolution, reduced scatter and decreased scan time can

be obtained by using an x-ray energy source instead of gamma rays[Augat, Fuerst, and

Genant 1998]. DXA technology projects the scanned volume of body tissue onto an area.

The attenuation of the x-rays is dependent on the type and amount of tissue in its path.

Taking soft tissue into account, the amount of bone mineral in the x-ray path may be

determined. The quantity of bone mineral is then determined per unit projected area of

bone in the region of interest. This gives an areal density measured in g/cm^2 and not a true

volumetric density.

The precision error and accuracy error of DXA measurements is good at the spine

(1-1.5% and 4-10%, respectively), hip (1.5-3% and 6%, respectively) and forearm (1%

and 5%, respectively)[Genant et al. 1996]. With the high precision and accuracy of DXA,

the low radiation dose (<2μSv effective dose)[Lewis, Blake, and Fogelman 1994] and

ease of use, DXA has become a popular choice for bone density measurement and

diagnosis of osteoporosis with an estimated 6,000 machines located worldwide in

1996[Genant et al. 1996; Grampp et al. 1997].

2.1.3 Peripheral Quantitative Computed Tomography

2.1.3.1   Measurement of Bone Mineral Content and Bone Mineral Density

The principles of pQCT are based upon QCT, but pQCT is dedicated to

measurement of peripheral sites, such as the distal radius. A diagrammatic representation

of measurement sites by pQCT and DXA is found on page 202. Quantitative computed

tomography takes a number of x-ray projections around a specimen and reconstructs an

image of a thin slice of the object. By quantifying the amount of x-ray attenuation in each

pixel, and using a conversion factor from a calibration phantom, bone content and density

may be determined[Augat, Fuerst, and Genant 1998]. Since QCT reconstructs the three-

dimensional object it scans by volumetric pixels (voxels), the amount of bone mineral

may be determined by volume. This allows for true volumetric bone density

measurements in mg/cm^3. Another advantage of three-dimensional evaluation is that the

cortical and trabecular bone compartments can be measured separately.

2.1.3.2 Separate Measurement of Cortical and Trabecular Compartments

The ability of pQCT to measure the cortical (outer shell) and trabecular (inner spongy bone) compartments separately (see FIGURE 2.1), is a great advantage over DXA. For example, it could be possible that while monitoring the radius, bone mass is being lost in one compartment while it is being gained in the other with no overall change in total bone mineral. A bone mass shift such as this could be detected with pQCT, but not with DXA. This has already been shown with bone changes due to short-term physical exercise. A recent study investigating the effects of a 6-month site-specific exercise regime in 250 postmenopausal women found no significant changes in BMD at the hip, spine or radius as measured by DXA between exercise and control groups [Adami et al. 1999]. This same study also measured bone density, content and area by pQCT for the trabecular, cortical and total bone compartments in the radius. No significant changes in any pQCT variable were found from baseline to six months for the control group. In the exercise group, however, cortical bone mineral content (BMC) increased 3.2% (p<0.01) and trabecular BMC was found to decrease 3.4% (p<0.05), but no significant changes were found in total BMC. This study demonstrates that pQCT was able to detect a shift of bone mass from the trabecular compartment to the cortical compartment at the distal radius that DXA could not detect. Furthermore, it has been shown that the ultra-distal radius is relatively non-responsive to treatment when measured by DXA[Bouxsein, Parker, and Greenspan 1999]. Future studies using pQCT may prove to discover shifts in bone mass from one compartment to another, or specifics of where

bone mass is accrued or lost, when investigating the effects of disease, disuse or treatment on the skeleton.

FIGURE 2.1

DIAGRAMS SHOWING CORTICAL AND TRABECULAR BONE AT THE DISTAL RADIUS IN LONGITUDINAL AND CROSS-SECTIONS

Close-up of trabecular structure

Longitudinal section of distal radius

Cortical shell

Cross-section of distal radius
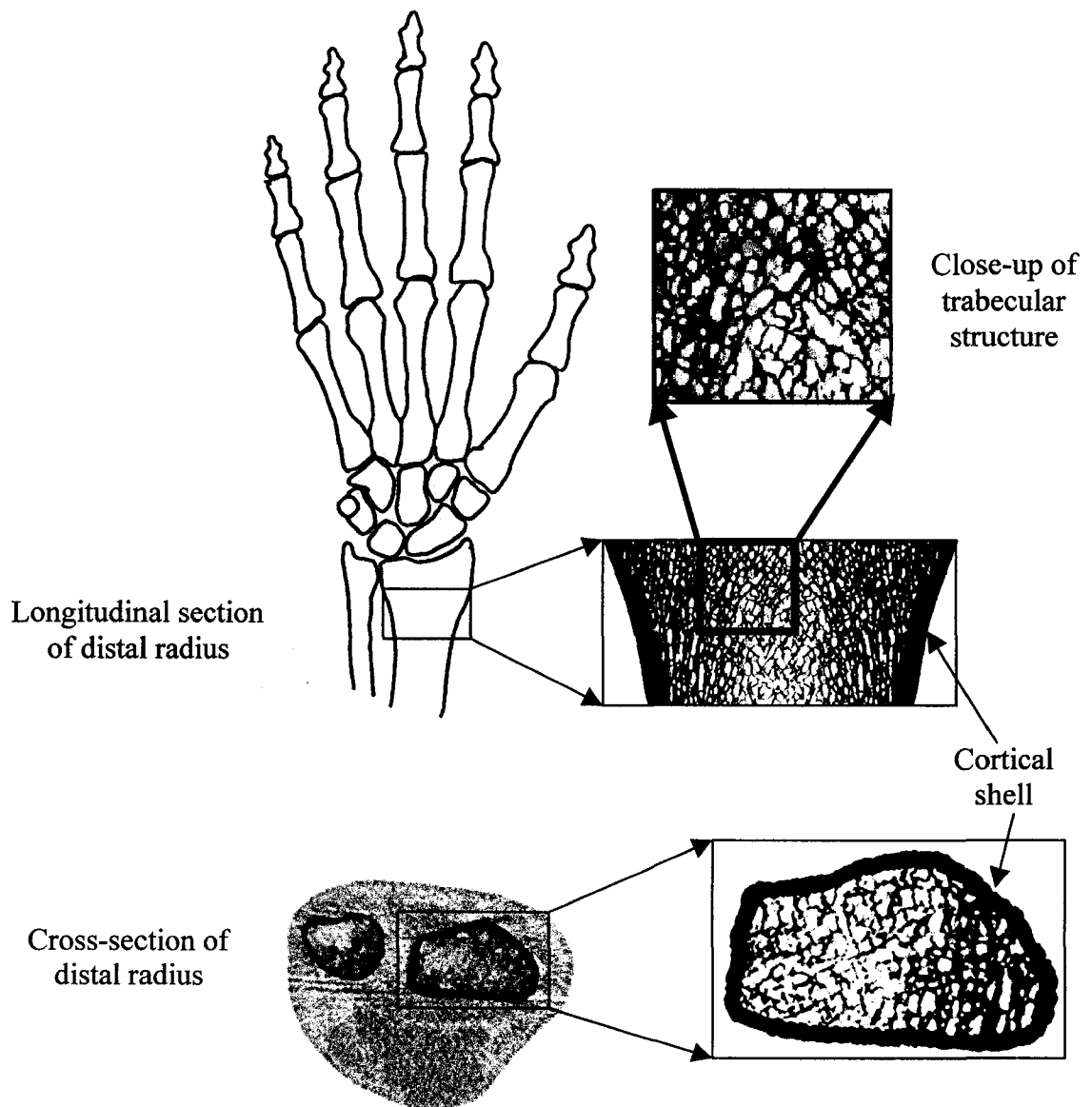
2.1.3.3   Measurement of Bone Geometry

Bone strength has been shown to depend not only on the amount of bone present, but also on the spatial arrangement of the bone material (see section 2.2). PQCT technology has allowed for the *in vivo* measurement of different bone geometry parameters at sites along the radius. A commonly reported geometric measure is cross-sectional area (CSA). Recent advancements in pQCT software have made it possible to measure other geometric properties such as cortical thickness, endocortical (inner side of the cortical shell) and periosteal (outer side of the cortical shell) circumference and a strength-strain index (SSI) which relates to bending and torsional strength of the bone[Norland Medical Systems Inc. 1999].

2.1.3.4   Measurement of Trabecular Bone Structure

Traditionally, trabecular bone structure has been measured using bone biopsy. Typically the biopsy is taken from the iliac crest, which is not a common site for fracture. Furthermore, the procedure is painful and invasive, and the same biopsy site can not be re-sampled to monitor changes with time, disease progression, or treatment[Muller et al. 1996]. Advancements in pQCT scanning have improved image resolution so that apparent trabecular structure may be detected[Gordon et al. 1996; Genant et al. 2000]. With specialized software developed for image processing, the trabecular architecture can then be quantified by various structural indices. The major limitations of pQCT in imaging trabecular structure are the smaller sampling sizes, larger doses of radiation and

longer scanning times needed to improve resolution[Genant et al. 2000]. This is not of

concern when bone samples are scanned, and very high-quality images can be

produced[Genant et al. 2000]. When scanning patients however, the need to restrict the

radiation dose and scanning time, and also to be able to image larger samples, limits the

attainable quality of the image. Working within these restrictions, it has still been possible

to achieve adequate resolution in order to collect meaningful trabecular structure

information *in vivo* using pQCT[MacIntyre, Adachi, and Webber 1999A; MacIntyre,

Adachi, and Webber 1999B]. Specifically, the expected gender differences, trends in

handedness and age-related changes in trabecular structure have been shown with *in vivo*

pQCT measurements[MacIntyre, Adachi, and Webber 1999A; MacIntyre, Adachi, and

Webber 1999B].

2.2   Bone Strength

When studying bone as a load-bearing structure, both cortical and trabecular bone

must be considered. Cortical or compact bone forms the shaft of long bones, known as the

diaphysis. The diaphysis is a hollow tube, containing bone marrow. As the cortical bone

extends towards the ends of a long bone, it narrows to a thin layer that covers the

metaphyseal and epiphyseal regions. Beneath this covering of cortical bone, within the

ends of the long bones, lies trabecular or spongy bone. Trabecular bone also constitutes

the greater part of vertebral bodies, deep to a thin cortical shell. Cortical bone is stiffer

than trabecular bone, but also more brittle. This means that cortical bone is able to

withstand higher stresses (force per unit area), but lower strains (changes in length per original length) before failure[Nordin and Frankel 1989].

Bone, as a functional weight-bearing structure, exhibits anisotropic properties, being stronger in one direction (primary loading direction) than another[Bouxsein, Courtney and Hayes 1995]. This anisotropy means that bone strength depends upon the mode and direction of loading. For example, bone tissue is stronger in a compressive than in a tensile mode of loading and vertebral bodies are stronger in a vertical than a transverse direction of loading[Mosekilde and Danielsen 1987; Ferretti 1997]. With weight bearing and muscle contraction, bones are placed under many different modes of stress in normal physiologic conditions, such as compression, tension, bending and torsion. The structural qualities of a bone will determine the degree of effectiveness in withstanding the different modes of loading. These structural qualities include not only the amount of bone material present (bone mass), but also the distribution of that bone material (geometry and architecture) and the quality of the bone material (intrinsic material quality and amount of microdamage or micro-cracks)[Ferretti, Schiessl, and Frost 1998]. The intrinsic material quality of bone and the degree of microdamage (microcracks) can be measured *in vitro* and are beyond the scope of this thesis. Advancements in bone measurement techniques have provided the means to non-invasively evaluate bone mass, bone geometry and trabecular structure *in vivo*. The *in vivo* measurement techniques have been discussed above in section 2.1; how the indices derived from the measurements relate to bone strength is discussed below.

2.2.1 Bone Mineral Density or Content and Bone Strength

Studies have shown that bone density measurements by ashing, DXA and QCT correlate well with bone strength measured *in vitro* for the hip and spine explaining most, but not all of the variance in strength (r^2= 0.75-0.86)[Mosekilde and Danielsen 1987; Bouxsein, Courtney and Hayes 1995; Ebbesen et al. 1999]. DXA measurements of BMD at the forearm, however, are controversial as predictors of radial bone strength. An *in vitro* study by Myers et al. found no significant correlation of forearm BMD with failure load while Wu et al. determined that forearm BMD explained approximately 50% of the variance in failure load(r^2=0.45-0.56) [Myers et al. 1993; Wu et al. 2000]. Bone mineral content at the forearm has also been slightly inconsistent as a predictor of bone strength, but generally, content is a better predictor of forearm compressive strength than density with correlations ranging from non-significant to r^2=0.62-0.89[Myers et al. 1991; Myers et al. 1993; Spadaro et al. 1994; Augat et al. 1998].

Bone density has also been shown to discriminate between fracture and non-fracture groups, but with a large overlap in individual values[Melton, Eddy, and Johnston 1990; Mallmin, Ljunghall, and Naessen 1992; Cummings et al. 1993; Greenspan et al. 1994; Lang et al. 2002]. Furthermore, BMD is able to moderately predict fracture risk[Marshall, Johnell, and Wedel 1996]. Studies evaluating the use of BMD measurements to predict fracture have shown that the best location to measure BMD is at the site of interest. For example, hip BMD is a better predictor of hip fracture (RR=2.6, CI_{95}=2.0-3.5) than spine BMD (RR=1.6, CI_{95}=1.2-2.2) and spine BMD is a better predictor of vertebral fracture

(RR=2.3, CI$_{95}$=1.9-2.8) than hip BMD (RR=1.8, CI$_{95}$=1.1-2.7)[Marshall, Johnell, and Wedel 1996]. Similarly, the forearm is the best site to measure BMD for prediction of forearm fracture (RR=1.7, CI$_{95}$=1.4-2.0; spine RR=1.5; hip RR=1.4). BMD at the distal radius has also been shown to be a moderate predictor of fracture at the hip (RR=1.8, CI$_{95}$=1.4-2.2) and spine (RR=1.7, CI$_{95}$=1.4-2.1)[Marshall, Johnell, and Wedel 1996].

Since there is a large overlap in fracture and non-fracture individuals and BMD is only a weak to moderate predictor of bone strength and future fracture, then other factors must play a role in bone strength and fracture. Some of these other factors are bone geometry, trabecular architecture and non-skeletal factors.

2.2.2 Bone Geometry and Bone Strength

From physics, the geometry of a structure is an important determinant of strength especially when considering loading in bending or torsion[Bouxsein, Myers, and Hayes 1996]. For example, bending strength is dependent on the moment of inertia, which is calculated from the amount of bone and from the *squared* distance of the bone from the central axis[Turner and Burr 1993]. Therefore a unit change in distance of a bone pixel from the axis will have a larger effect on bone bending strength than a unit change in bone mass. The importance of bone geometry in predicting mechanically tested bone strength at the hip and distal radius has been shown *in vitro* [Myers et al. 1993; Bouxsein, Courtney, and Hayes 1995; Augat, Reeb, and Claes 1996]. Specifically, Myers et al. and Augat et al. both found that moment of inertia and areal measures were better predictors of failure load of the distal radius than measures of BMD, explaining around 50-70% of

the variance in bone strength[Myers et al. 1993; Augat, Reeb, and Claes 1996].

Furthermore, *in vivo* research has demonstrated the importance of bone geometry in predicting fracture and in discriminating between fracture and non-fracture subjects independently of BMD[Faulkner et al. 1993; Gnudi et al. 1999]. Following over 8000 women for an average of a year and a half, Faulkner et al. found that a 1 SD increase in a hip geometric parameter (hip axis length) nearly doubled the risk of hip fracture even after adjustment for age, BMD, height and weight[Faulkner et al. 1993].

2.2.3   Trabecular Bone Structure and Bone Strength

As shown in section 2.2.1 bone density explains a large portion but not all of the variance in bone strength. Bone architecture has been investigated as a determinant of bone strength and *in vitro* studies have found significant correlations of trabecular structure with mechanically tested bone strength at the spine, hip and radius[Vesterby et al. 1991; Link et al. 1998; Gordon, Webber, and Nicholson 1998; Jiang et al. 1998]. Structure indices that significantly predicted strength explained 20-45% of the variance in bone strength at the proximal femur, 30-60% at the spine and 50-75% at the radius [Vesterby et al. 1991; Link et al. 1998; Gordon, Webber, and Nicholson 1998; Jiang et al. 1998]. Some studies have demonstrated that structure measurements improve the prediction of bone strength above that of BMD measures alone[Link et al. 1998; Gordon, Webber, and Nicholson 1998; Jiang et al. 1998]. At the spine, prediction of bone strength improved from $r^2=0.66$ for BMD alone to $R^2=0.80$ (p<0.01) when structure was added, the hip was similar with an increase from $r^2=0.61$ to $R^2=0.72$ (p<0.01) when a structure

measure was added to density[Link et al. 1998]. In one study structure measurements at the distal radius improved the prediction of bone strength from r^2=0.76 (BMD alone) to R^2=0.86 (p<0.01)[Jiang et al. 1998]. A study mechanically testing excised distal radii found trabecular bone density alone explained 54% of the variance in bone strength, but with the addition of a measure of the maximum hole size, the correlation increased to R^2=0.63[Gordon, Webber, and Nicholson 1998]. Addition of a second structural parameter, average hole size, further increased the predictive ability to R^2=0.83[Gordon, Webber, and Nicholson 1998]. These results emphasize the importance of trabecular architecture and suggest that structural indices add additional information to BMD for the determination of bone strength.

Trabecular structure parameters have also demonstrated the ability to discriminate between fracture and non-fracture patient groups[Kleerekoper et al. 1985; Gordon et al. 1998; MacIntyre, Adachi, and Webber 2003]. When 26 patients with vertebral fracture were compared with 24 women without vertebral fracture who were matched for age, menopausal status and bone density, trabecular structure indices were able to discriminate between the two groups[Kleerekoper et al. 1985]. Trabecular plate density, thickness and separation of iliac bone biopsies were found to be significantly different between the two groups suggesting that trabecular structure is an important determinant of fracture[Kleerekoper et al. 1985]. *In vivo* measurement of vertebral trabecular structure using QCT has also shown the ability to distinguish between fracture and non-fracture subjects[Gordon et al. 1998]. At the spine, Gordon et al. found that even though all

structural indices were significantly correlated with BMD, after adjustment for BMD, average hole size continued to significantly discriminate between fracture and non-fracture groups[Gordon et al. 1998].

2.2.4   A Note on Biomechanical Behaviour of Bone In Vivo

Although many biomechanical studies have shown good predictive value of bone strength by measured bone characteristics, it must be remembered that fractures in physiologic conditions occur under much more complex conditions than are simulated *in vitro*. Bone strength studies load bones in the absence of muscle forces and usually without soft tissue cushioning. Furthermore, most fractures *in vivo* do not occur under a single loading mode, but usually a complex combination of multiple loading modes from muscle contraction and weight-bearing, which would be extremely difficult to duplicate[Nordin 1989].

CHAPTER 3: SKELETAL EFFECTS OF PHARMACOLOGICAL TREATMENTS FOR OSTEOPOROSIS

3.1 Background

3.1.1 Pharmacological Treatment of Osteoporosis

There have been many different pharmacological agents tested in the treatment of

osteoporosis including: calcium, vitamin D, hormones, fluoride, bisphosphonates,

calcitonin and SERMs (selective estrogen receptor modulators). This study focuses on the

effects of three anti-resorptive agents, alone or in combination, on the spine, hip and

distal radius. These are estrogen and two bisphosphonates, etidronate and alendronate.

3.1.2 Clinical Research

The majority of clinical studies measuring the effects of treatment for

postmenopausal osteoporosis on BMD or fracture, focus on the hip and spine. Clinically,

the hip is considered a very important site for measurement since hip fractures cause the

highest morbidity, mortality and economic burden[Karpf et al. 1997]. Therefore,

measurements of BMD and fracture at the hip give important site-specific information.

The lumbar spine, however, appears to be more sensitive to pharmacological treatments

for osteoporosis than the hip or forearm, showing the greatest percentage changes in

BMD (see section 3.2 below). Furthermore, the spine is the most common site of osteoporotic fracture. Vertebral fractures, therefore, are used as the primary endpoint in many fracture studies in order to try keep the large sample size needed in these types of trials as low as possible[Karpf et al. 1997]. Although many vertebral fractures are asymptomatic, these fractures can be detected radiographically, which is the method of detection used in clinical trials.

In this thesis, change in bone mass at the distal radius is the primary endpoint (the rational for choosing the radius as a measurement site has been already discussed in section 1.3.3). DXA measurements of the spine and hip were also included in this study in order to compare these results with previous research to assess whether the BMD changes in these study groups are comparable to those seen in large randomized controlled trials.

3.2   Overview of Previous Studies

3.2.1   Treatment Effects on BMD

There are many different pharmacological agents available for the prevention and treatment of postmenopausal osteoporosis. The medications prescribed in this study include hormone replacement therapy and two bisphosphonate drugs, alendronate and etidronate. All of these medications have been shown to improve bone mineral density over time. Since this thesis involves the measurement of bone mineral density and bone

mineral content, previous studies measuring bone mineral density or content are reviewed in the following sections.

3.2.2 Treatment Effects on Fracture

BMD is really measured as a surrogate of bone strength, since bone strength can not be measured *in vivo*. Although BMD has been shown to predict the occurrence of osteoporotic fractures[Marshall, Johnell, and Wedel 1996], it is still not the most meaningful outcome measure since the ultimate goal of osteoporosis treatment is not to increase BMD but to reduce fractures. For this reason, studies regarding the effects of treatment on fractures are also reviewed. It should be noted that where the spine is concerned, many trials report changes in vertebral fracture rate (i.e. number of vertebral fractures per 'x' patient years). Vertebral fracture rate is not the proper statistical measure since it assumes that each fracture within a patient is independent, which is not the case[Meunier et al. 1999]. Therefore, the appropriate measure should be the number of patients with one or more new vertebral fractures. This is taken into consideration when reviewing the literature.

3.2.3 Hormone Replacement Therapy

3.2.3.1 Effects of HRT on Lumbar Spine and Proximal Femur BMD

The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial was a relatively large randomized, double-blinded, placebo-controlled clinical trial enrolling 875 healthy postmenopausal women aged 45 to 64 years (with any baseline BMD value)[The Writing

Group for the PEPI Trial 1996]. Of the subset of women ≥ 65 years of age who had a

baseline BMD measurement, less than 15% were found to be osteoporotic (i.e. T-score

≤2.5). Subjects were assigned to either placebo or one of four active treatment protocols

including 0.625mg of conjugated equine estrogens (CEE) alone or in combination with

various progestin formulations. After one year of therapy active treatment groups showed

significant gains in BMD at the lumbar spine from baseline of 3.0-3.6%, while the

placebo group lost 1.4% (treatment effects of 4.4-5.0%). After three years, active

treatment groups had gained 3.5-5.0% in lumbar spine BMD, while the placebo group

lost 1.8% (treatment effects of 5.3-6.8%)[The Writing Group for the PEPI Trial 1996].

These gains with HRT were significant using both intention-to-treat analysis and valid

case analysis. BMD at the hip was significantly higher in women taking active therapy

than the placebo group at both the one and three year time points. The placebo group

realized losses of around 1.7% by three years, while HRT treatment groups gained around

1.7% at the hip giving a 3.4% treatment effect (all sites at the hip were reported as similar

and data was not shown) [The Writing Group for the PEPI Trial 1996]. In a randomized

controlled trial of 107 women over 65 years old with low bone mass, Recker et al.

reported similar significant gains at the lumbar spine from baseline at three years of 4.0%

in subjects taking 0.3mg of CEE with progestin (treatment effect of 4.4%)[Recker et al.

1999]. Hip BMD, although higher in the treatment group at every time point, did not

significantly differ from the placebo group. The HRT group gained approximately 2.5%

in femoral neck BMD (treatment effect of around 2.2%)[Recker et al. 1999] The Danish

Osteoporosis Prevention Study (DOPS) enrolled 2016 recently postmenopausal women without a vertebral fracture (and any baseline BMD) and measured BMD by DXA at the spine, hip and forearm[Mosekilde et al. 2000]. The 5-year DOPS report showed a slightly larger treatment effect for HRT at the spine (approximately 8%) than the PEPI trial, but these subjects were followed for 2 more years than the women in the PEPI trial. Although subjects on HRT in DOPS may have lost a small amount of BMD at the hip (-0.3%, p=0.20), they found a similar treatment effect of 3.2% at the hip compared to the PEPI trial. Lufkin et al. enrolled 75 postmenopausal women (mean age 65 years) with $\geq$ 1 vertebral fracture (mean spine BMD $0.78g/cm^2$) in their RCT with dermal HRT patches and found slightly larger, significant BMD gains of 5.3% at the lumbar spine and 2.6% at the femoral neck over baseline after one year of therapy[Lufkin et al. 1992].

The same group that published a meta-analysis on the effects of etidronate therapy on postmenopausal osteoporosis performed a similar meta-analysis evaluating the effects of HRT on postmenopausal osteoporosis[Wells et al. 2002]. Pooled estimates of BMD gains over placebo for the lumbar spine were 5.4% (CI_{95} 4.2 to 6.5) and 6.8% (CI_{95} 5.6 to 7.9) at one and two years, respectively. At the femoral neck, BMD gains of 2.5% (CI_{95} 1.2 to 3.8) and 4.1% (CI_{95} 3.4 to 4.8) were found at one and two years, respectively versus placebo[Wells et al. 2002]. The trial by Recker et al. found slightly smaller gains than the meta-analysis, which may be due to this study using a lower dose of estrogen (0.3mg/day of CEE) versus the more commonly used 0.625mg/day (of CEE)[Recker et al. 1999].

PEPI sub-analyses determined that older women gained significantly more BMD from baseline than younger women and that women with lower baseline BMD gained more BMD than women with higher baseline BMD values[The Writing Group for the PEPI Trial 1996].The trial by Lufkin et al. (1992) and more recent trials enrolling older women with lower baseline BMD values support the findings of the PEPI sub-analyses. Harris et al. followed 524 postmenopausal women (mean age in HRT group of 59.8 years) in a double blind, placebo-controlled study and found significant one-year gains over baseline in lumbar spine and femoral neck BMD of 4.6% and 1.8%, respectively with HRT[Harris et al. 2001]. Delmas et al. enrolled 135 postmenopausal women (mean age of 58 years) in a 2-year double blind placebo-controlled study and found that the two HRT groups gained an average of 5.3% (treatment effect of 6.2%) at the spine[Delmas et al. 2000]. Bone density gained at the femoral neck, however, was lower at 0.7% (CI_{95} -1.3 to 2.8) in one HRT group and 1.5% (CI_{95} -0.5 to 3.4) in the other HRT group, neither being significant versus placebo (treatment effects of 1.7% (p=0.18) and 2.5% (p=0.06), respectively)[Delmas et al. 2000].

3.2.3.2 Effects of HRT on Forearm BMD

Results are somewhat mixed when considering BMD changes at the radius with HRT therapy. Different studies have found no change, significant decreases or significant increases in radial BMD with HRT. The study by Recker et al. measured forearm BMD by DXA and found that the HRT group gained 1.2% over 3.5 years which was significant when compared to the 0.8% loss of the placebo group (treatment effect of 2.0%)[Recker

et al. 1999]. Similar to Recker et al., some other studies have also reported significant

treatment effects of HRT on BMD at the radius without finding significant increases in

radial BMD in the HRT groups versus baseline values. A smaller randomized controlled

trial using SPA and examining the effects of transdermal HRT patches over one year, also

reports a significant treatment effect of HRT on bone mass at the radius relative to the

placebo group, but no significant gain in the HRT group from baseline[Lufkin et al.

1992]. Lufkin et al. found a non-significant gain in mid-radius of 1% with HRT and a

significant loss of -2.6% with placebo patches. The treatment effect of 3.6% was

significant[Lufkin et al. 1992]. In two thousand recently postmenopausal women,

Mosekilde et al. showed that both the HRT and non-HRT groups may have lost bone

mineral density at the ultradistal radius, but there was a significant difference between

groups, with the HRT group losing significantly less bone than non-HRT

group[Mosekilde et al. 2000]. Some studies have found significant changes in radial

BMD over baseline values. A long-term study by Eiken et al. found a significant decrease

of 0.7% in bone mass at the forearm measured by SPA over 10 years of treatment with

HRT[Eiken, Kolthoff, and Nielsen 1996]. The placebo group, however, showed a 17.6%

decrease in bone mass at the forearm over 10 years, equating to a 16.9% treatment effect

of HRT[Eiken, Kolthoff, and Nielsen 1996]. This study enrolled 151 women with only

42% (64 subjects) of women finishing the 10 years of follow-up. The subjects were early

postmenopausal women (6-24 months after last menstruation), with any baseline BMD,

but without vertebral fracture. A possible reason for the decrease in bone mass in the

treatment group, but still a positive treatment effect could be due to the young age and early postmenopausal status of the subjects.

The meta-analysis by Wells et al. (mentioned in the above section), also examined changes in forearm BMD with HRT versus control. Twenty of the studies included in the meta-analysis measured forearm BMD. The pooled estimates for BMD gains at the forearm with HRT over control were 3.0% (CI_{95} 2.3 to 3.7) at one year and 4.5% (CI_{95} 3.7 to 5.4) at two years[Wells et al. 2002]. The recent two year double blind, placebo controlled RCT by Delmas et al. in 135 postmenopausal women found increases of 0.9-2.1% in the distal radius with HRT[Delmas et al. 2000]. The treatment effects found by Delmas et al. at the distal radius (treatment effect of 1.6-2.8%) were slightly smaller than reported by the meta-analysis. The women in the Delmas et al. study had baseline BMD T-scores of -2 to +2 and this could help explain the smaller percentage gains[Delmas et al. 2000]. Since these women did not have osteoporosis and were closer to their peak bone mass, then similar absolute gains would appear as smaller percentage gains. A recent randomized, placebo-controlled and double-blinded study by Harris et al. on 524 postmenopausal women (HRT vs HRT + risedronate), found a significant increase of 1.7% at the distal radius at one year with HRT alone[Harris et al. 2001]. Since a non-treatment group was not included in this trial, the treatment effect could not be calculated[Harris et al. 2001]. As with the Delmas et al. study, the women in this study were generally not osteoporotic and the mean baseline lumbar spine T-score was

-1.4[Harris et al. 2001]. For the same reasons as mentioned above, this may help to explain the smaller forearm BMD gains than was found in the meta-analysis.

3.2.3.3 Effects of HRT on Fracture

Many observational studies have supported the conclusion that HRT reduces fracture[Meunier et al. 1999; Hochberg 2000]. One prospective randomized controlled trial has investigated the effects of transdermal estrogen on bone in 75 postmenopausal women with one or more vertebral fracture[Lufkin et al. 1992]. This study showed that transdermal HRT reduced fracture compared to the placebo group. However, fracture rate and not the number of patients with new fractures was used in their analysis. Re-analysis by the patient with fracture method showed no statistically significant difference in fracture risk between the HRT treated and placebo groups[Meunier et al. 1999]. Recently there have been a few other randomized prospective trials examining the effect of HRT on fracture reduction. Komulainen et al. enrolled 464 postmenopausal women from Kupio in Finland and followed the subjects for 5 years on one of four treatments, HRT, vitamin D, HRT plus vitamin D or placebo (calcium)[Komulainen et al. 1998]. In this study, they found that HRT alone significantly reduced non-vertebral fracture risk relative to the placebo group by 62-71%. When the two groups taking HRT were pooled, treatment significantly reduced non-vertebral fracture risk by 56-63%[Komulainen et al. 1998]. No significant reductions in fracture risk were seen for the vitamin D group alone.

Mosekilde et al. reported on the five-year results of the DOPS[Mosekilde et al. 2000]. Results were similar for the randomized (n=1006) and non-randomized arm

(n=1010) and therefore these were analyzed together. With intention to treat analysis

HRT was found to significantly reduce forearm fractures by 55% and showed a trend to

reduce all fractures by 27% (p=0.09). When causal analysis (including only women who

stayed with their original treatment or non-treatment group) was performed, significant

reductions of 76% in forearm fractures and 39% in all fractures were seen[Mosekilde et

al. 2000]. The reduction in forearm fractures in the women taking HRT was not paired

with an increase in forearm BMD measured by DXA. In fact, the HRT subjects had a

small but significant decrease in forearm BMD. This discordance in BMD changes and

fracture reduction suggests that there may be other effects occurring with HRT to reduce

the incidence of fracture. These other effects could be shifts in bone mass from one

compartment to another and/or changes in trabecular structure which would not be

detected with DXA, or non-skeletal benefits, such as a reduction in fall

tendency[Mosekilde et al. 2000]. The utilization of pQCT could potentially detect some

of these skeletal changes at the forearm.

Recent meta-analyses of randomized trials have evaluated the effect of HRT on both

non-vertebral and vertebral fractures[Torgerson and Bell-Syer 2001A; Torgerson and

Bell-Syer 2001B; Wells et al. 2002]. In their first meta-analysis, Torgerson and Bell-Syer

focussed on non-vertebral fractures and included studies up until the end of 2000. The

pooled analysis of the 22 trials included in their report found an overall 27% reduction in

non-vertebral fractures (RR=0.73, CI_{95} 0.56 to 0.94, p=0.02) by HRT[Torgerson and

Bell-Syer 2001]. The second meta-analysis by Torgerson and Bell-Syer focussed on

vertebral fractures and included 13 randomized controlled trials up until August 2001.

They found a significant reduction in vertebral fractures of 33% (RR=0.67, CI_{95} 0.45 to

0.95) by HRT[Torgerson and Bell-Syer 2001]. The meta-analysis by Wells et al. included

6 RCT trials and found a smaller, non-significant reduction in non-vertebral fractures

with HRT of 13% (RR=0.87, CI_{95} 0.71 to 1.08)[Wells et al. 2002]. Wells et al. also found

a trend towards a 34% reduction in vertebral fractures in a pooled estimate of 5 RCT

trials (RR=0.66, CI_{95} 0.41 to 1.07)[Wells et al. 2002]. Although published later than the

Torgerson and Bell-Syer meta-analyses (2001, 2001A), the report by Wells et al. (2002)

only searched the literature until 1999 and included fewer trials, but all were RCTs. The

previously mentioned primary studies and these meta-analyses provide moderate to strong

evidence of both vertebral and non-vertebral fracture risk reductions with HRT.

3.2.4   Etidronate

3.2.4.1   Effects of Etidronate on Lumbar Spine and Proximal Femur BMD

A recent meta-analysis searched MEDLINE from 1966-1998 and included 13

randomized studies of postmenopausal women treated with etidronate or control (placebo

or calcium and/or vitamin D) for at least one year[Cranney et al. 2001]. Data was pooled

for years 1-3 for each separate body site measured. Ten trials (n=875) included

measurements of lumbar spine BMD by DPA or DXA. The weighted mean percent

difference of lumbar spine BMD between treatment and control groups after 1-3 years

was +4.06 for the treatment group (CI_{95} 3.12 to 5.00, p<0.01)[Cranney et al. 2001].

There were 8 studies (n=800) reporting hip BMD. The meta-analysis found a treatment

effect of 2.35 (CI_{95} 1.66 to 3.04, p<0.01) at the femoral neck for 1-3 years. Four-year

data was analyzed separately and treatment effects were 10.1 and 5.7 (p<0.01 for both)

for the spine and hip, respectively.

Since the meta-analysis by Cranney et al., there have been a small number of

randomized trials involving etidronate and a control group[Heath et al. 2000; Adami et al.

2000; Shiota et al. 2001]. These trials have found results similar to Cranney et al., but

with a larger range than the 95% confidence intervals of the meta-analysis. The more

recent studies found significant, positive treatment effects on BMD at the lumbar spine of

1.6%, 2.8% and 16.3% at two years[Heath et al. 2000; Adami et al. 2000; Shiota et al.

2001]. The smaller treatment effects of 1.6-2.8% were found in women who were 6-36

months postmenopausal and are comparable to the results of other prevention studies

found in the meta-analysis[Heath et al. 2000; Adami et al. 2000; Cranney et al. 2001]. In

general, the prevention studies consistently reported smaller gains than the treatment

studies included in the meta-analysis[Cranney et al. 2001]. The recent study by Shiota et

al. found a very large gain in lumbar spine BMD with etidronate (+10.2%) and a

significant loss of BMD in the control group (-6.1%). This large treatment effect of over

+16% may be due in part to racial differences (Japanese subjects) and also due to the fact

that their study population had very low mean baseline BMD of 0.560 g/cm^2 and 0.574

g/cm^2 for the etidronate and control groups, respectively[Shiota et al. 2001]. This means

that similar absolute gains and losses in BMD will become much larger as percent gains

and losses in this population with very low baseline BMD compared to the previous trials with higher mean baseline densities[Shiota et al. 2001; Cranney et al. 2001]. Adami et al. and Heath et al. also reported etidronate effects on the femoral neck. Adami et al. found a 0.06% loss (not significant), but a significant +2.2% treatment effect, while Heath et al. reported a +3.2% treatment effect over the two years, values which are similar to the treatment effect in the meta-analysis[Heath et al. 2000; Adami et al. 2000].

3.2.4.2   Effects of Etidronate on Forearm BMD

The meta-analysis by Cranney et al. included four trials that measured forearm bone density or content[Cranney et al. 2001]. The four trials enrolled 368 women in total and together showed a weighted mean difference of 1.11% in forearm BMD for etidronate versus control (CI_{95} -1.16 to 3.38), which was not significant (p=0.34)[Cranney et al. 2001]. A recent randomized controlled trial in Japan enrolled 72 women who were 53-78 years old and at least 5 years postmenopausal and measured forearm BMD by DXA[Iwamoto, Takeda, and Ichimura 2001]. After one year, the etidronate group had significantly gained 2.2% over baseline while the control group had lost 2.4%. This translated into a treatment effect of 4.6% for etidronate. After two years, the etidronate group was virtually unchanged (2.1% over baseline) and the control group that was taking calcium had lost 1.7% versus baseline. The treatment effect at two years was 3.8%[Iwamoto, Takeda, and Ichimura 2001]. The slightly larger treatment effect may be related to cultural differences or a lower baseline BMD.

3.2.4.3 <u>Effects of Etidronate on Fracture</u>

The recent meta-analysis by Cranney et al. also examined the effects of etidronate

on vertebral and non-vertebral fracture[Cranney et al. 2001]. Nine of the included studies

reported on vertebral fractures with a total of 1076 postmenopausal women, but three

studies were not included in the pooled estimate due to the low incidence of fractures

(n=315). Cranney et al. reported the results to be consistent among the remaining six trials

(n=716) and that etidronate reduced vertebral fracture risk by 37% (pooled estimate of the

relative risk was 0.63 (CI_{95} 0.44 to 0.92, p=0.02))[Cranney et al. 2001]. A recent study by

Iwamoto et al. enrolled 72 postmenopausal osteoporotic women and found a reduction in

the risk of new vertebral fractures by 68% for etidronate versus control[Iwamoto, Takeda,

and Ichimura 2001]. The reported risk reduction is larger than that of the meta-analysis,

but this trial also found a larger treatment effect on forearm BMD with etidronate. Likely

the larger gain in BMD and the greater reduction in fracture risk are linked. Conversely,

non-vertebral fracture risk was not found to be reduced by etidronate therapy in the meta-

analysis by Cranney et al.[Cranney et al. 2001]. Seven studies (n=867) reported non-

vertebral fracture data, but one was not included (n=80) due to zero fractures occurring.

The pooled estimate of the relative risk of non-vertebral fractures for the six studies

(n=787) was 0.99 (CI_{95} 0.69 to 1.42)[Cranney et al. 2001].

One caution with the Cranney et al. meta-analysis is that the pooled estimate for

fracture risk likely used the fracture rate and not the number of patients with new

fractures method. Three trials using the fracture rate method found significant reductions

with etidronate therapy versus placebo[Watts et al. 1990; Storm et al. 1990; Harris et al. 1993]. However, inconsistent results were found when the number of patients with fracture method was used. Watts et al. showed a significant reduction in fracture risk with etidronate treatment at 2 years[Watts et al. 1990]. When this same study was continued for a third year, significant reductions in fracture risk were no longer evident[Harris et al. 1993]. Furthermore, a four year trial in 72 postmenopausal women evaluating etidronate alone, or in combination with HRT, found no significant change in fracture risk with etidronate treatment relative to placebo[Wimalawansa 1998].

At present, there is no randomized, placebo controlled, double-blinded study using the number of patients with new fracture method that has shown, with sufficient power, that etidronate reduces fracture risk. Three reviews have found that although etidronate increases bone density, there is no conclusive evidence that this drug reduces fracture risk[Meunier 1999; Hochberg 2000; Marcus et al. 2002].

3.2.5 Alendronate

Alendronate was developed after etidronate and is a nitrogen-containing bisphosphonate that is a more potent inhibitor of bone resorption than its predecessor. The effects of alendronate on bone have been well investigated, and high quality evidence exists for both BMD and fracture. Some of the main research studies are the FIT (Fracture Intervention Trial)[Black et al. 1996; Cummings et al. 1998; Hochberg et al. 1999; Black et al. 2000], FOSIT (Fosamax International Trial)[Schneider et al. 1999; Pols et al. 1999], EPIC (Early Postmenopausal Intervention Cohort)[Hosking et al. 1998] and

the American and international trials from the Alendronate Phase III Osteoporosis Treatment Study Group[Liberman et al. 1995; Tucci et al. 1996; Devogelaer et al. 1996; Tonino et al. 2000].

3.2.5.1 Effects of Alendronate on Lumbar Spine and Proximal Femur BMD

In a large one-year study of 1908 postmenopausal women with low bone mass at the lumbar spine, 10mg of alendronate daily was found to significantly improve lumbar spine BMD by 5.0% over baseline[Pols et al. 1999].

Liberman et al. reported on a 3-year study of alendronate that showed gains from baseline in lumbar spine BMD of around 5%, 7% and 8% for 1, 2 and 3 years of treatment, respectively in the 10mg daily group [Liberman et al. 1995]. Women taking 5mg daily of alendronate had significantly less bone gain than the 10mg group and measured 4-5% increases at 1, 2 and 3 years. The portion of the FIT involving 2027 women with low BMD and at least one vertebral fracture at baseline, found significant increases at the lumbar spine at year one of 4-5%, at year two of just over 6% and at year three of around 8%. In the first two years of this study, patients receiving alendronate took only 5mg/day, which was increased to 10mg daily for the third year[Black et al. 1996]. The arm of the FIT following 4432 postmenopausal women with low bone mass, but without vertebral fracture at baseline, showed percentage gains in lumbar spine BMD for each of years 1, 2, 3 and 4 of around 4%, 5-6%, 7% and over 8%, respectively. As in the vertebral fracture arm of FIT, women taking alendronate received 5mg daily for the first two years followed by 10mg/day thereafter[Cummings et al. 1998].

Similar to HRT and etidronate therapy, alendronate produced slightly smaller gains in BMD at the proximal femur than at the lumbar spine. After one year of treatment, Pols et al. reported BMD increases from baseline of 2.3% at the femoral neck[Pols et al. 1999]. Liberman et al. found significant increases in BMD at the hip after three years of 10mg daily alendronate of around 5% versus baseline at the femoral neck[Liberman et al. 1995]. The FIT reported increases in femoral neck BMD of 3.8% after 3 years for women who entered the vertebral fracture arm of the study and a similar increase in femoral neck BMD for women who entered the non-vertebral fracture arm of the study, but had taken alendronate for 4 years[Black et al. 1996; Cummings et al. 1998].

The same group that published the meta-analyses on etidronate and HRT also performed a meta-analysis of alendronate for the treatment of postmenopausal women[Cranney et al. 2002]. Their results give a pooled estimate of a 7.5% (CI_{95} 6.1 to 8.8) increase in lumbar spine BMD over placebo with $\geq$10mg/day of alendronate over 2-3 years of treatment. Hip BMD increased 5.6% (CI_{95} 4.8 to 6.4) with $\geq$10mg/day of alendronate over 3-4 years of treatment relative to placebo[Cranney et al. 2002].

3.2.5.2   Effects of Alendronate on Forearm BMD

Measurements concerning the forearm are generally less common since there is more clinical importance placed upon the hip and spine. Forearm BMD measurements were not performed in all of the large randomized controlled trials; some studies only measured forearm BMD in a subset of the total subjects and not all measurement scans were taken at the same site. Devogelaer et al. reported the results of the international arm

of the Phase III trials, which included forearm measurements at both the 1/3-distal site and ultradistal site with DXA on all of the subjects[Devogelaer et al. 1996]. After three years of treatment with 10mg/day of alendronate, BMD increased 0.6% at the 1/3-site and 1.7% at the ultradistal site, although neither reached significance. When looking at the treatment effect, however, forearm BMD was significantly greater in the 10mg treatment group than the placebo group by 2.6% at the 1/3-site and 3.4% at the ultradistal site at three years[Devogelaer et al. 1996]. The combined Phase III trials showed a significant treatment effect of 2.2% over placebo at the mid forearm at three years[Liberman et al. 1995]. In the FIT, only 20% of the subjects were measured at the forearm, yet this still included a large number of subjects (around 400 subjects in the vertebral fracture arm and nearly 900 women in the non-fracture arm of the trial) [Black et al. 1996; Cummings et al. 1998]. Significant treatment effects were found in both arms of the FIT. In the vertebral fracture arm, proximal forearm BMD had significantly increased 1.5% versus placebo at 3 years[Black et al. 1996]. In the non-vertebral arm of the trial, 1/3-distal BMD of the forearm had significantly increased 3.1% versus placebo over the 4 years of follow-up[Cummings et al. 1998].

The meta-analysis by Cranney et al. included most of the large studies as well as some smaller randomized controlled trials and found a pooled estimate of a 2.1% (CI_{95} 1.5 to 2.6) increase in forearm BMD over placebo with 2-4 years of ≥10mg of alendronate[Cranney et al. 2002].

Of significant interest to this thesis is a paper by Schneider et al. reporting on a sub-study of the FOSIT[Schneider et al. 1999]. Out of the 1908 subjects enrolled in FOSIT, 103 subjects were enrolled in a sub-study, which included scanning at the forearm with pQCT during the yearlong trial. As in the main study, these women were postmenopausal for at least 3 years and had lumbar spine BMD of at least 2 standard deviations below the mean of the young adult female. The subjects were randomized into placebo or 10mg daily alendronate for one year. All subjects were given 500mg/day of calcium. PQCT measurements of total and trabecular bone density were taken at the ultradistal radius (4% site) by a Stratec XCT-900 scanner. Lumbar spine and femoral neck BMD were also measured by DXA to assess the response to treatment. The significant gains in BMD ($p<0.001$) at the spine (5.7%) and hip (3.4%) at one-year were in line with, but slightly greater than, the results of the larger RCTs (spine gains of 4-5% and hip gains of 2-3%)[Schneider et al. 1999]. The treatment group also realized a significant gain versus baseline for total bone density (6.3%, CI_{95} 1.48 to 11.04), but not trabecular bone density (5.3%, CI_{95} -4.26 to 14.83) at the distal radius. Treatment effects were significant at the distal radius after 12 months. Total density increased 6.8% versus placebo ($p=0.009$) and since the significance level was set at $p < 0.10$, the treatment effect in trabecular density at the ultradistal site was reported to be significant as well, with an increase of 8.4% versus placebo ($p=0.095$)[Schneider et al. 1999].

3.2.5.3 Effects of Alendronate on Fracture

Good quality and consistent evidence exists for both vertebral and non-vertebral

fracture reduction with alendronate. In the Phase III trials combined, new radiographic

fractures of the spine were decreased by 48% relative to placebo over three

years[Liberman et al. 1995]. Similar reductions in vertebral fractures were seen in the

vertebral fracture arm of the FIT (47% reduction over 3 years) and the non-vertebral

fracture arm of the FIT (44% reduction over 4 years)[Black et al. 1996; Cummings et al.

1998]. A combined analysis of all women with osteoporosis (defined by either the

presence of vertebral fracture at baseline or T-score of less than −2.5 but without vertebral

fracture) from both arms of FIT also showed a reduction in new radiographic vertebral

fractures of 48% versus placebo over 3-4 years[Black et al. 2000]. Reductions in clinical

vertebral fractures in FIT ranged from 45-55% versus placebo over 3-4 years[Black et al.

1996; Cummings et al. 1998; Black et al. 2000]. The meta-analysis by Cranney et al.

found similar results with a 48% reduction (RR=0.52, CI$_{95}$ 0.43 to 0.65) in vertebral

fractures in women given ≥ 5mg of alendronate[Cranney et al. 2002].

When considering non-vertebral fractures, Pols et al. found significant reductions of 47%

after only one year of treatment with 10mg daily alendronate versus the placebo

group[Pols et al. 1999]. Non-vertebral fractures were reduced around 12-20% in the

Phase III trials and both arms of FIT, but did not reach statistical significance in any of

these studies[Liberman et al. 1995; Black et al. 1996]. In the combined FIT analysis

including only osteoporotic women, statistically significant reductions in non-vertebral fractures of 27% were found over 3-4 years versus placebo[Black et al. 2000].

Some studies examined the influence of alendronate treatment on specific non-vertebral fractures. For instance, in the FIT hip fractures were reduced in the treatment group of the vertebral fracture arm of the FIT by 51% over 3 years relative to placebo[Black et al. 1996]. There was a 21% reduction in hip fracture with treatment when all subjects were considered for analysis in the non-vertebral arm of FIT, but this was not statistically significant. In post-hoc analysis, however, a subset of approximately 1500 women in the non-vertebral arm, who had a femoral neck BMD T-score of -2.5 or less, showed a 56% reduction in hip fractures with treatment[Cummings et al. 1998]. Furthermore, the combined analysis of osteoporotic women from both arms of FIT showed a similar reduction of 53% with alendronate versus placebo[Black et al. 2000]. These results from FIT suggest a gradient effect, where women with lower BMD(T-score ≤ -2.5) gain more anti-fracture benefit than those with higher BMD.

Treatment effects on wrist fracture are controversial. After 3 years Liberman et al. found wrist fractures in 16 (4%) women in the placebo group versus 8 (1.3%) in the treatment groups, but this greater than 50% reduction was not significant, likely due to the small sample size[Liberman et al. 1995]. In the FIT, the vertebral fracture arm found a significant 48% reduction in wrist fractures with treatment versus placebo[Black et al. 1996]. Conversely, the non-vertebral fracture arm of FIT reported no significant reduction in wrist fracture incidence in the treatment groups versus the placebo group. In fact, in the

subset of subjects with higher baseline BMD (T-scores greater than −2.0 at the femoral neck), forearm fracture was almost doubled in the treatment versus placebo group (increase of 90%)[Cummings et al. 1998]. When the combined analysis was performed on the subset of osteoporotic women in FIT (T-score less than −2.5 without vertebral fracture or presence of vertebral fracture) however, there was a 30% reduction in wrist fracture with treatment over 3-4 years versus placebo[Black et al. 2000]. Again, this data suggests a gradient effect, where women with lower BMD have better fracture reduction benefits than women who have higher BMD scores at baseline. Karpf et al. performed a meta-analysis of 5 prospective randomized, double blinded, placebo controlled studies at least 2 years in length(including the Phase III study), which had enrolled women with lumbar spine BMD T-score of ≤ −2.0, and found a significant risk reduction of wrist fractures of 61% over three years[Karpf et al. 1997].

The meta-analysis by Karpf et al. also found that alendronate significantly reduced non-vertebral fractures by 29% over placebo[Karpf et al. 1997]. The more recent meta-analysis by Cranney et al. found a 49% reduction (RR=0.51, CI_{95} 0.38 to 0.69) in non-vertebral fractures in patients given ≥ 10mg of alendronate, which they stated, was a larger effect than the 5mg dose[Cranney et al. 2002].These meta-analyses allow for pooling of results to increase the number of subjects, which is important since non-vertebral fracture studies need very large numbers of subjects due to the low incidence of these fractures. Most importantly, the results of these meta-analyses support the trends and findings from the other studies mentioned above.

3.2.6 Combination Therapy

Some studies have investigated the combination of HRT with a bisphosphonate to determine if there are any additional skeletal benefits of pairing the medications. The theory for combining these two antiresorptive agents is that estrogen and bisphosphonates have different mechanisms of action and therefore may be able to exert a greater effect on bone mass and fracture rate together, than either agent alone[Recker and Heaney 2001]. As well, many women whose bone mass has not responded to estrogen may continue to take the hormone for the extra-skeletal benefits on menopausal symptoms, but also add a bisphosphonate to improve skeletal strength. Another benefit of combination therapy may be the reduction of side-effects associated with larger doses of any one medication, since combining therapies may allow for the administration of lower doses of each individual drug. Areas of concern regarding the combination of two antiresorptives include additional side-effects, increased cost to the patient and greater reduction of remodeling rates, which may affect repair[Recker and Heaney 2001]

3.2.6.1   Effects of Combination Therapy on BMD

Two studies by Wimalawansa looked at the additive effects of taking etidronate and HRT on BMD. The first study enrolled 58 early postmenopausal women who were randomly allocated into an HRT (transdermal patch), etidronate, HRT (patch) plus etidronate or a control group[Wimalawansa 1995]. Each subject also received 1000mg of calcium. Lumbar spine DXA results showed significant increases of 3.7% and 4.2% at 2 years for the HRT alone and etidronate alone groups and an increase of 6.8% (for both

groups) at 4 years. The combination therapy group gained significantly more BMD ($p<0.05$) at the spine than either single therapy with increases of 6.4% at 2 years and 10.9% at 4 years. The control group lost 2.3% and 3.8% at 2 and 4 years. BMD changes at the femoral neck showed similar patterns with combination therapy gaining significantly more ($p<0.01$) density than either of the single therapies. The HRT plus etidronate group gained 4.5% and 7.2% at 2 and 4 years, respectively, while HRT increased 2.4% and 4.0% and etidronate increased 1.5% and 1.2%. Group comparisons at the hip also determined that the HRT alone group gained significantly more density than the etidronate alone group, suggesting HRT is more effective at increasing BMD at the hip than etidronate in early postmenopausal women. Women in the control group lost 3.2% and 5.0% at the hip at 2 and 4 years, respectively[Wimalawansa 1995].

The second study enrolled 72 postmenopausal women who had at least 1, but not more than 4, vertebral fractures and had a lumbar spine BMD at least two standard deviations below the young normal value[Wimalawansa 1998]. Women were randomized into HRT, etidronate or HRT plus etidronate treatment groups or the control group. The control group significantly lost bone from baseline at the lumbar spine and total hip at 2 and 4 years, while all treatment groups significantly gained bone at both sites at 2 and 4 years. At the lumbar spine, the combination therapy group (etidronate plus HRT) gained significantly more bone from baseline at 2 years and 4 years (6.4% and 10.4%, respectively) than either of the single therapy groups (around 4% gain and 7% gain for 2 and 4 years, respectively for both groups). The combination therapy group also gained

significantly more bone at the total hip than the etidronate group at 2 and 4 years and more than the HRT group at 4 years. The HRT group gained more bone (4.8%) at the total hip than the etidronate group (0.9% gain) after 4 years, again suggesting that etidronate is less effective at increasing bone density at the hip than HRT[Wimalawansa 1998].

A few recent trials have studied the effects of the combination of alendronate and HRT on bone mass[Lindsay et al. 1999; Tiras et al. 2000; Bone et al. 2000]. These trials have been slightly shorter than the etidronate combination therapy trials with two trials of one year duration and one trial of two years duration. Lindsay et al. administered alendronate or placebo to 428 postmenopausal women with low bone mass (T-score <-2) already taking HRT for at least one year[Lindsay et al. 1999]. At 12 months significant gains were seen for the lumbar spine of 1.0% and 3.6% for HRT and HRT plus alendronate groups, respectively, while at the femoral neck corresponding gains were 0.8% and 1.7%[Lindsay et al. 1999]. At the lumbar spine and trochanter, significant differences were seen between groups at 6 months and 12 months with the combination therapy group (alendronate plus HRT) gaining more bone from the start of the study than the subjects who continued to take HRT by itself. The femoral neck showed similar trends, but did not reach statistical significance (p=0.072 between groups at 12 months). Another one-year study randomized 120 postmenopausal women with low bone mass to HRT, alendronate or combination therapy (HRT plus alendronate) groups[Tiras et al. 2000]. This study had no placebo or control group, was not blinded and did not include

data from 21 subjects who were deemed to have low compliance. All treatment groups gained significant bone from baseline at the spine and femoral neck. Similar to Lindsay et al., at the lumbar spine, the combination therapy group gained significantly more bone from baseline than the HRT group at 6 months (4.8% versus 1.3%) and 12 months (8.4% versus 2.6%)[Tiras et al. 2000]. Gains in spine BMD with alendronate were intermediate at 4.0% and 7.2% for 6 and 12 months, respectively, which were significantly greater than the gains with HRT alone. No significant differences were seen between any treatment groups at the femoral neck, but there was a trend for combination therapy to increase BMD more than either HRT or alendronate alone. Femoral neck BMD gains were 3.0% for alendronate, 3.2% for HRT and 4.6% for combination therapy at 12 months[Tiras et al. 2000]. In 2000, Bone et al. published a well designed trial including HRT and alendronate and their combination[Bone et al. 2000]. This trial was a randomized, placebo-controlled, double-blinded study of 425 postmenopausal women who had undergone a hysterectomy and had low bone mass. Data was collected over 2 years and intention to treat analysis was used. The placebo group had no significant changes in BMD over 2 years at either the spine or the hip. At the lumbar spine, the HRT and alendronate groups had significant increases of 6.0% each from baseline, but the combination therapy group gained significantly more than either of these groups with an increase of 8.3% from baseline[Bone et al. 2000]. A similar pattern was seen at the femoral neck with combination therapy gaining significantly more bone (4.2% gain) from

baseline than either HRT or alendronate alone (2.6% and 2.9% gains, respectively)[Bone et al. 2000].

In general, all monotherapies (HRT, etidronate, or alendronate alone) evaluated in parallel with the combination therapies in the studies discussed above had similar effects on BMD at the spine and hip to those found in the RCTs described in previous sections.

3.2.6.2 Effects of Combination Therapy on Fracture

No published combination therapy trials have been large enough or long enough to determine any significant effects on fracture risk.

3.2.7 Effects of Treatment on Structure

As mentioned previously in chapter 2 section 2.1.3.4 trabecular structure is traditionally assessed from bone biopsies of the iliac crest using histomorphometric techniques, but recently, developments in high-resolution computed tomography have allowed non-invasive, *in vivo,* measurement of trabecular architecture in humans[Gordon et al. 1996]. Studies evaluating the effects of antiresorptive medications on bone structure *in vivo* in postmenopausal women have not, to my knowledge, been reported in the literature. One study has been published, which evaluates the effect of HRT on bone microstructure in postmenopausal women by utilizing bone biopsies and histomorphometry[Vedi et al. 1996]. This study analyzed iliac crest bone biopsies from 22 women with osteopenia (n=5) or osteoporosis (n=17) at baseline and after a mean of two years of HRT. No placebo or control group was included. The results showed no

statistically significant differences between baseline and post-treatment structural indices, nor were there any consistent trends to indicate either an improvement or decline in architectural integrity suggesting that HRT is able to preserve trabecular structure in postmenopausal women for two years[Vedi et al. 1996]. Without a control group, however, these results should be interpreted with caution since the two year duration of the trial may not have been long enough for an untreated postmenopausal women to develop changes in trabecular structure that could be detected using these same techniques.

Due to the invasiveness of bone biopsies and ethical considerations, it would be unlikely for histomorphometric studies to contain a placebo or control group. Furthermore, the same site cannot be resampled after treatment and biopsies are traditionally taken from the iliac crest, which is a non-weightbearing site. For these reasons, *in vivo* assessment by high-resolution computed tomography or MRI presently appear to have the most potential for measuring treatment-induced changes in trabecular structure at the same site as compared to a placebo group.

3.2.8 Summary of Treatment Effects on BMD at the Hip and Spine

HRT has shown BMD gains at the lumbar spine of 3-5.3% at one year and 3.5-6% by 2-3 years and femoral neck changes of -0.3% (p=0.02) to 2.6% by 1-5 years[Lufkin et al. 1992; Wimalawansa 1995; The Writing Group for the PEPI Trial 1996; Wimalawansa 1998; Recker et al. 1999; Delmas et al. 2000; Mosekilde et al. 2000; Bone et al. 2000; Harris et al. 2001]. Treatment effects for HRT are slightly larger at 4.4-5.4% for one year

and 4.4-6.8% at 2-3 years at the lumbar spine and 1.4-2.5% at one year and 2.2-5.6% at 2-5 years for the femoral neck[Lufkin et al. 1992; Wimalawansa 1995; The Writing Group for the PEPI Trial 1996; Wimalawansa 1998; Recker et al. 1999; Delmas et al. 2000; Mosekilde et al. 2000; Bone et al. 2000; Wells et al. 2002]

Etidronate trials have reported BMD changes from baseline of 2-5% at the lumbar spine at 2-3 years and -0.06% (not significant) to 2% at the femoral neck by 2-3 years[Watts et al. 1990; Storm et al. 1990; Wimalawansa 1995; Herd et al. 1997; Wimalawansa 1998; Adami et al. 2000]. The meta-analysis by Cranney et al. reported BMD gains as treatment effects and found etidronate to increase lumbar spine BMD by 3-5% over control and femoral neck BMD by 1.5-3% over control in 1-3 years[Cranney et al. 2001].

Alendronate has been shown to produce the largest BMD gains of the three individual anti-resorptive drugs included in this thesis. At the lumbar spine alendronate has produced gains of 4-5%, 5-7% and around 8% by 1, 2 and 3-4 years, respectively[Liberman et al. 1995; Black et al. 1996; Cummings et al. 1998; Pols et al. 1999; Bone et al. 2000]. Increases in femoral neck BMD with alendronate therapy have been reported at around 2-3%, 2.5-3.5% and 4-5% by 1, 2 and 3-4 years, respectively[Liberman et al. 1995; Black et al. 1996; Cummings et al. 1998; Pols et al. 1999; Bone et al. 2000].

Combination therapies have generally produced larger gains in BMD than any of the individual therapies[Wimalawansa 1995; Wimalawansa 1998; Lindsay et al. 1999; Tiras

et al. 2000; Bone et al. 2000]. Etidronate plus HRT produced 6.4% gains at the lumbar spine and 4.6% gains at the femoral neck after two years of treatment[Wimalawansa 1995; Wimalawansa 1998]. Alendronate plus HRT produced slightly larger increases in BMD at the lumbar spine of around 8-8.5% after 1-2 years and similar gains as etidronate plus HRT therapy at the hip with increases of 4.2-4.6% at the femoral neck after 1-2 years of treatment[Tiras et al. 2000; Bone et al. 2000].

In general, etidronate produced the smallest gains in BMD, followed by HRT, then alendronate, with combination therapy showing the largest gains in bone density.

3.2.9 Summary of Treatment Effects on BMD at the Radius

Gains in forearm BMD with HRT are inconsistent, but generally are 1-2% over baseline by 1-3 years of treatment[Lufkin et al. 1992; Recker et al. 1999; Delmas et al. 2000; Harris et al. 2001]. Occasionally studies report losses in forearm BMD with HRT, but these drops in density in the treatment groups are still less than the amount of bone lost in the control or placebo groups, leading to positive treatment effects[Eiken, Kolthoff, and Nielsen 1996; Mosekilde et al. 2000]. The meta-analysis by Wells et al. estimated larger treatment effects in forearm BMD with HRT of 3% at one year and 4.5% at two years[Wells et al. 2002]. The larger treatment effects estimated in the meta-analysis could be due to the inclusion of many smaller HRT trials that may have reported greater increases in forearm BMD than were found in some of the larger RCTs reviewed in this chapter.

The meta-analysis on etidronate by Cranney et al. estimated a 1% treatment effect over 1-3 years, but this was not significant (p=0.34)[Cranney et al. 2001]. The studies reviewed in this chapter found similar results, except for a recent RCT by Iwamoto et al. in 72 postmenopausal osteoporotic Japanese women[Iwamoto, Takeda, and Ichimura 2001]. This trial found approximately 2% gains at 1-2 years with etidronate, but this variance may be due to cultural differences or a lower baseline BMD[Iwamoto, Takeda, and Ichimura 2001].

Alendronate effects on forearm BMD have generally been reported as treatment effects. BMD increases at the forearm with alendronate treatment relative to placebo have been reported as 1.6-2.6% after 3 years and 3.1% after 4 years[Liberman et al. 1995; Devogelaer et al. 1996; Black et al. 1996; Cummings et al. 1998] . The meta-analysis by Cranney et al. supports this finding with a pooled estimate of a 2% treatment effect[Cranney et al. 2002]. The international arm of the Phase III trials reported forearm BMD gains of 1.7% versus baseline, but these were not significant[Devogelaer et al. 1996].

The combination therapy trials to date, have not evaluated forearm BMD.

3.2.10 Summary of Effects of Treatment on Fracture

There are many observational studies and a meta-analysis that strongly suggest positive effects of HRT on fracture reduction, however, HRT is lacking a large, prospective, randomized, placebo-controlled, double-blind clinical trial that definitively shows fracture risk reduction. The DOPS found a trend for a 27% reduction in all

fractures and the meta-analysis by Torgerson et al. reported a 27% reduction in non-vertebral fractures[Mosekilde et al. 2000; Torgerson and Bell-Syer 2001A]. Mosekilde et al. also reported a significant 55% reduction in wrist fractures in the DOPS[Mosekilde et al. 2000]. Analyzing only the women who continued with their original treatment, DOPS found a significant reduction of 39% in all fractures and 76% in forearm fractures[Mosekilde et al. 2000].

Etidronate is also lacking a definitive large, prospective, randomized, placebo-controlled, double-blind clinical trial showing fracture risk reduction. The published meta-analysis by Cranney et al. suggests that etidronate does reduce vertebral fracture risk but not non-vertebral fractures. It is unclear, however, if the proper number of new patients with fractures method is used in their analysis[Cranney et al. 2001].

Alendronate has been shown to reduce the risk of vertebral and non-vertebral fractures in large randomized placebo controlled trials. Vertebral fracture risk has been reduced by 44-48% over 3-4 years of treatment with $\geq$ 5mg of alendronate[Liberman et al. 1995; Black et al. 1996; Cummings et al. 1998; Pols et al. 1999; Black et al. 2000]. Alendronate has been shown to reduce non-vertebral fracture risk by 47% in one year and 27% over 3-4 years in postmenopausal women with T-scores of $\leq$ -2.0[Pols et al. 1999; Black et al. 2000]. The meta-analysis by Karpf et al. also found a 29% reduction in non-vertebral fractures. Considering specific non-vertebral fractures, hip fractures were reduced by 53% over 3-4 years by alendronate in women with lower BMD (T-score $\leq$ -2.5) in the combined analysis of the FIT trials, which was similar to the 54% reduction

found in the meta-analysis by Karpf et al, but this reduction was not significant

(p=0.15)[Karpf et al. 1997; Black et al. 2000]. Wrist fractures were reduced by 30% in

the osteoporotic women of the FIT trials and by 61% in the meta-analysis by Karpf et al.

[Karpf et al. 1997; Black et al. 2000].

Neither combination therapy (HRT plus etidronate or HRT plus alendronate) has

shown proven fracture risk reduction.

3.3   Relationship of BMD Gain to Fracture Risk Reduction

Although the ultimate goal of treatment for osteoporosis is to reduce fracture, these

are infrequent events that require large RCTs to determine efficacy of a therapy. The

surrogate measure of bone density has therefore been adopted for many of the smaller

trials and also in this thesis. There have been a few analyses of individual trials and

groups of RCTs that have investigated the relationship between BMD gains and fracture

risk reductions with antiresorptive treatments[Hochberg et al. 1999; Wasnich and Miller

2000; Hochberg et al. 2002]. Hochberg et al. (1999) evaluated BMD and vertebral

fracture rate changes with alendronate in 2984 women in the FIT who were at least two

years postmenopausal and had low femoral neck BMD ($\leq 0.68 g/cm^2$)[Hochberg et al.

1999]. They found that women who gained the most BMD (at the total hip or lumbar

spine) had the lowest incidence of new vertebral fractures, while women with the lowest

gains in BMD had the highest fracture rate. This suggests that the magnitude of vertebral

fracture risk reduction is associated with the magnitude of increase in BMD. The next

year, Wasnich and Miller published a meta-analysis of BMD and vertebral antifracture

efficacy in RCTs of antiresorptive agents[Wasnich and Miller 2000]. Their results

supported those of Hochberg et al. as they also found that greater increases in BMD were

associated with larger reductions in vertebral fracture risk[Hochberg et al. 1999; Wasnich

and Miller 2000]. For example, a 4% gain in spine BMD gave a 38% reduction in

vertebral fracture rate, whereas an 8% gain in spinal BMD gave a 54% reduction in

vertebral fracture incidence[Wasnich and Miller 2000]. In their analyses, Wasnich and

Miller also determined that there were treatment effects on vertebral fracture reduction

that were independent of BMD gains. Some of the proposed mechanisms for BMD

independent vertebral fracture reduction include the effects of changes in bone turnover

on remodelling coupling, response to mechanical stimuli, trabecular structure and

mineralization[Wasnich and Miller 2000; Hochberg et al. 2002]. Specifically focussing

on trabecular structure, some suggest that reducing the rate of bone turnover preserves

trabecular bone strength by reducing the number of full trabecular perforations[Riggs,

Khosla, and Melton 2002]. Preventing the loss of horizontal supporting trabeculae and

more subtle changes such as filling in the remodeling space leading to a reduction in the

number of resorption pits have also been proposed[Parfitt 2002]. Parfitt (2002) considers

these resorption pits to be focal structural weaknesses that could lead to increased bone

fragility by buckling under force. Therefore a reduction in the number of resorption pits

by reducing the bone turnover rate may reduce fracture risk beyond that of BMD gains

alone[Parfitt 2002].

A recent meta-analysis evaluated antiresorptive treatments and the association of BMD changes with nonvertebral fracture incidence[Hochberg et al. 2002]. This meta-analysis also determined that larger gains in BMD are associated with larger reductions in fracture risk, this time, nonvertebral fractures. One difference that was found between the association of BMD gains with nonvertebral fracture risk versus the association with vertebral fracture risk was that there was no significant effect of treatment on nonvertebral fracture risk that was independent of BMD. A reason for this contrast could be due to the proportions of trabecular and cortical bone at each site, with the vertebral site containing primarily trabecular bone, whereas the hip and other non-vertebral sites generally contain more cortical bone. Trabecular bone is much more dependent on architectural integrity than cortical bone is for its' strength. Trabecular bone also has a higher rate of turnover than cortical bone. Therefore, treatments reducing bone turnover would likely have a larger effect in trabecular bone strength than cortical bone strength, and would presumably have a greater effect on the spine than the hip[Riggs, Khosla, and Melton 2002]. BMD independent treatment effects on fracture reduction (due to alterations in trabecular structure characteristics) would therefore be more prominent at the spine and other trabecular sites than at the hip or other primarily cortical sites.

3.4 Thesis Objectives

1 - To determine the effects of different antiresorptive treatments, alone or in combination, on bone at the distal radius, lumbar spine and proximal femur in postmenopausal women with low bone mass.

Specifically:

> ➢ Bone mass and geometry changes in the total, trabecular and cortical bone compartments at the distal radius with treatment, as measured by pQCT

> ➢ Bone density changes at the hip and spine with treatment, as measured by DXA

> ➢ Trabecular structure changes at the distal radius with treatment, as measured by pQCT and specialized structure analysis software.

2 - To determine which clinically available bone measurement, or combination of measurements, is the best predictor of *in vitro* bone strength at the distal radius.

CHAPTER 4: METHODS

4.1 Subjects

4.1.1 Subject Selection

Subjects for this study were postmenopausal women with low bone mass or

evidence of radiographic vertebral fracture. Low bone mass was defined as osteopenia or

osteoporosis in either the hip or spine by DXA measurement, according to WHO

guidelines (i.e. a DXA T-score of less than 1 SD below the young adult mean value at the

hip or spine)[Kanis et al. 1997]. Subjects were considered postmenopausal if at least six

months had passed since their last menstruation or if their serum FSH levels were greater

than 40mIU/mL. In the event of a hysterectomy (without oophorectomy), subjects had a

blood test to determine baseline serum FSH levels (see section 4.1.5.1). Subjects with

FSH levels above 40 IU/mL were considered postmenopausal and eligible for the study,

while women with serum FSH below 40 IU/mL were excluded. Each subject gave

informed written consent.

4.1.2 Subject Exclusion Criteria

Reasons for exclusion were as follows: evidence of disease (other than osteoporosis)

or use of therapies that affect calcium metabolism, use of any medications for

osteoporosis within the 3 months prior to study entry, the inability to keep their

measured arm immobile for the full 10 minute forearm scan and lack of postmenopausal status.

4.1.3 Subject Recruitment

The majority of the subjects were recruited from a metabolic bone disease clinic of a local Rheumatologist, Dr. JD Adachi. Dr Adachi is affiliated with St. Joseph's Hospital and McMaster University and his clinic is at 25 Charlton St. W., room 501. Twelve subjects were recruited from a previous study in our laboratory at McMaster University. These subjects did not have baseline blood testing (see section 4.1.5.1).

4.1.4 Calcium

4.1.4.1 Dietary Calcium Assessment and Supplementation

A brief dietary questionnaire was given to determine whether the patient required calcium supplementation. Subjects with daily calcium intake estimated below 400mg were advised to take supplements[Dawson-Hughes et al. 1990]. Sufficient calcium carbonate for 500mg daily (in 250mg tablets) was supplied to these subjects to last until the next visit in six months time. Subjects who could not take calcium carbonate were advised to try other supplements such as calcium citrate. Any patient with high serum calcium or renal disease was advised against calcium supplementation by the physician.

4.1.5 Non-Bone Baseline Measurements

Each subject's date of birth, date of menopause (if available), and date of

hysterectomy and/or oophorectomy (if applicable) were recorded. Height and weight were

measured, and any prescription medication was noted.

4.1.5.1 Blood Tests

All of the subjects recruited from the metabolic bone disease clinic had serum

measurements of Vitamin D (25-hydroxy-vitamin D), calcium, phosphate and alkaline

phosphatase to ensure normal or adequate baseline levels. Vitamin D supplementation

was prescribed to those subjects whose serum level was below the lower limit of normal

(16 ng/mL). If subjects were within six months of their last menstruation or had

undergone a hysterectomy without an oophorectomy, and were less than 60 years of age,

serum FSH was measured. Women with serum FSH levels of greater than 40 mIU/mL

were considered postmenopausal.

4.2 Treatment Groups

4.2.1 Treatment Groups

Subjects enrolling in this study were either taking no therapy for osteoporosis, or

were commencing prescribed medication in one of the following five active treatment

regimens: hormone replacement therapy (HRT), alendronate (ALN), etidronate (ETD),

HRT plus alendronate, and HRT plus etidronate. HRT was prescribed as 0.625mg of

conjugated equine estrogen per day, and for subjects with an intact uterus, 2.5-5.0mg of

medroxyprogesterone per day. Alendronate was prescribed as 10mg per day, taken immediately in the morning with water, one half to two hours before any food or other drink. Etidronate has a cyclic dosage regime starting with 14 days of 400mg of etidronate, followed by a 76 day no-dose period, in which 500mg of calcium per day is taken.

The prescription of pharmaceutical treatment for osteoporosis was based on physician recommendation following case assessment and consideration of the patient. For example, both the expert medical opinion of the physician and the personal concerns of the patient were considered when determining the best choice for pharmaceutical intervention. Subjects were given the initial six weeks to establish whether or not they wished to continue on the prescribed medication. Treatment group was determined by the medication that was being taken at the scheduled 6-week check-up point.

4.3   Bone Measurements

4.3.1   Types of Measurements

Bone measurements were performed using two different techniques. The first is dual-energy x-ray absorptiometry (DXA), the standard on which the WHO based their definition of osteoporosis. This technique is reviewed in section 2.1.2 of this thesis. The amount of bone corresponds to the attenuation of the x-ray sources. Bone content and density are measured within different regions of interest. DXA projects a three-dimensional object into two dimensions, and therefore, measures of bone density are not

true volumetric densities (amount of bone per volume), but areal densities (amount of bone per area). Typically DXA measurements are taken at the hip and lumbar spine.

Peripheral quantitative computed tomography (pQCT) was the second technique utilized in this study (see section 2.1.3). PQCT measures a transverse slice of bone in the periphery, specifically in this study, a slice at the distal radius. Similar to DXA, the amount of bone corresponds to the attenuation of the x-ray source, however the bone content is measured in a given volume, and therefore, a true volumetric density is measured by pQCT. Scanning with pQCT also allows for the measurement of the trabecular and cortical bone compartments separately, as well as a measurement of the total slice.

4.3.2 Measurement Schedule

Subjects were measured at baseline and once per year for two years by DXA giving a total of three scans. PQCT measurements were more frequent, and subjects were scanned every six months over two years yielding five measurements.

4.3.3 Peripheral Quantitative Computed Tomography(pQCT)

4.3.3.1 Measurement Procedure

The non-dominant wrist was measured in each subject. Subjects who had previously broken their non-dominant wrist were measured on their dominant side. The length of the subject's forearm was measured from olecranon process to ulnar styloid. The pQCT scanner (Stratec XCT-960, Norland Corporation, Fort Atkinson, WI, USA) utilizes a fan

beam from a 38 keV x-ray tube which produces a low patient dose of 0.02

mSv[MacIntyre 1999]. The high-resolution pQCT measurement includes a five-minute

scout scan followed directly by a five-minute measurement scan. The scout scan produces

a 30mm coronal view of the distal radius and ulna. The scout view allows visual

assessment for positioning of the scanning location for the measurement scan. The

operator places a reference line at the proximal aspect of the distal articular surface of the

radius. The software automatically locates the measurement site proximal to the reference

line by 4% of the forearm length. Using the translate-rotate principle, 145 projections are

acquired around the forearm during the measurement scan. Reconstruction of these

projections produces an image of the transverse slice at the 4% site of the forearm. The

slice thickness is 2.5mm, and using high-resolution software (xmice, v1.0), the in-plane

pixel size is 0.33mm by 0.33mm and allows for apparent trabecular structure analysis.

4.3.3.2 Radial Bone Mass and Geometry Variables

Separation of the soft tissue from the outer bone edge and of the subcortical and

cortical bone from the inner trabecular bone was performed using an operator-

independent function in the pQCT software. This method of segmentation into trabecular

and cortical (including subcortical bone) compartments divides all of the voxels of the

total bone into either the trabecular or the cortical compartment, aiding in the detection of

shifts in bone mass from one compartment to another. In other words, there are no

portions of the total bone that are unaccounted for when considering the trabecular and

cortical compartments together.

Bone characteristics were measured for the total (TOT), trabecular (TRAB) and combined subcortical/cortical (CRT) compartments. Measurements were made for bone amount and geometry. The variables measured for each compartment were volumetric bone density (vBD) in mg/cm3, cross-sectional area (CSA) in mm2 and bone content (CNT) in mg for the measured slice. The xmice software determined volumetric bone density for TOT, TRAB and CRT compartments. The software analysis also gives cross-sectional area for each bone compartment. From the vBD and CSA measurements, bone content was calculated for the 2.5mm slice at the 4% distal radial site for each bone compartment.

4.3.3.3   Trabecular Structure Analysis

Trabecular bone structure at the 4% distal radial site was evaluated using a software program developed in our laboratory. The pQCT images were saved to disk and transferred to a Sun workstation (Sun Microsystems, Mountain View, CA, USA) for processing and analysis. Structure variables considered the size(s) of the holes in the trabecular network as well as an estimation of how well the trabecular architecture is connected. The three structure variables that were derived from this software are average/mean trabecular pore size (Ha), maximum trabecular pore size (Hm), and connectivity index (C.I.) as described by Gordon et al. in 1996[Gordon et al. 1996].

4.3.4 Dual-energy X-ray Absorptiometry(DXA)

Measurements were taken at the femoral neck (FN) of the hip and at the lumbar

spine (LS) for each subject according to the manufacturer's protocol. The bone density

technician working with each individual DXA machine performed the measurements. It

should be noted that since this study is evaluating treatment effects on bone in a clinical

setting, DXA measurements were used from the original clinic at which each subject was

measured. A total of 10 different clinics and 13 different machines were used in this

study. Each subject, however, was scanned each year on the same machine. Therefore,

prospective changes in BMD from baseline may be compared at the 12 and 24 month

marks. Baseline data, however, are compared as T-scores since clinical decision making

is based on individual T-scores from different DXA machines and subjects in this study

were treated based on these decisions.

4.4   Statistical Analysis

The data analyses were performed using Minitab (release 13.20, State College, PA,

USA) commercial statistical software and some ANOVA testing was performed using

SPSS (release 10.0.5, Chicago, IL, USA) commercial statistical software. Data analysis

for this study was divided into two main sections. One section is focussed on the baseline

data and the second section evaluates the prospective data. For both sets of analyses, the

total cohort was divided into different treatment regimens according to different criteria.

4.4.1 Division of the Total Cohort by Different Treatment Criteria for Analysis

The statistical analysis was approached considering three separate questions aimed at examining the effects of anti-resorptive treatments as a whole, separately and in combination. First, what are the effects of anti-resorptive treatments on bone at the distal radius and are they different than the effects of no treatment? Secondly, are the effects of treatment on the radius different between subjects taking one anti-resorptive treatment versus two anti-resorptive treatments versus no treatment? Lastly, are there any differences between the effects of each of the six individual treatment regimens?

In order to answer these questions, the whole cohort was separated into groups by different criteria before analyses were performed. The first criteria for division of subjects is based on whether the subject is or is not taking any therapeutic agent for prevention or treatment of osteoporosis. Subjects taking any of the three anti-resorptive medications prescribed in this study are allotted to the 'Any Treatment' group, subjects who are not taking any of these agents are considered in the 'Control' group. The second criteria for division separates the cohort into three groups, subjects taking two anti-resorptive drugs versus subjects taking only one anti-resorptive drug versus subjects taking no medication. Those subjects taking no medication are in the 'Control' group, subjects taking one anti-resorptive treatment (ALN, ETD or HRT) are in the '1-Tx' group and women prescribed two anti-resorptive medications (A+H or E+H) are in the '2-Tx' group.

The final division of the total cohort is into all six of the individual treatment groups: five active therapy groups (A+H, ALN, E+H, ETD or HRT) and a control group (CNTL).

These same strategies for division of the total cohort are used for description of the baseline data and analysis of the prospective data. Therefore, for each analysis, the whole group of subjects is divided into 2 groups ('Any Treatment' or 'Control'), 3 groups ('1-Tx', '2-Tx' or 'Control') and into 6 groups (A+H, ALN, CNTL, E+H, ETD or HRT).

4.4.2   Baseline Data

4.4.2.1   Initial Data Management

To help identify data entry errors, retrospective examination of data was performed after all subjects had completed their 5th and final visit at the 2-year time point. Subject inclusion, pQCT and DXA data were inspected. PQCT and DXA data were examined for outliers and these values were double-checked for accurate entry into the spreadsheet.

Missing data points were replaced by the last available data point for that subject (i.e. last value carried forward technique). If a baseline measurement was missing, then data for that specific measurement was not included in the analysis.

Tests for normality were performed for all pQCT bone density measurements for the total, trabecular and cortical compartments. The normality testing was performed on the whole cohort and each separate group after division into 2, 3 and 6 different treatment groups as described in section 4.4.1.

4.4.2.2   Descriptive Statistics

Information and measurements from all subjects entering the study were described

as a total cohort of 123 subjects, and after division into different groups according to

treatment regimens. To determine if any differences existed between groups at baseline,

two-tailed t-tests (for division into two groups) or one-way ANOVA (for division into

more than two groups) were performed with significance set at $p<0.05$.

4.4.2.3   Comparison of Measurement Techniques to Detect Osteoporosis

The ability of different measurement techniques to differentiate between

osteoporotic and osteopenic individuals was assessed using data collected at baseline. The

z-statistic was used along with the WHO definitions for osteoporosis and osteopenia. The

techniques evaluated were: DXA of the hip (T-score), DXA of the spine (T-score), pQCT

volumetric density and content measures of the distal radius for total, cortical and

trabecular compartments, pQCT geometric measures of cross-sectional area for all three

bone compartments, and pQCT trabecular structure measurements of Ha, Hm and

connectivity index.

4.4.3   Prospective Data

4.4.3.1   Initial Data Management

Data was analyzed using the intention to treat principle, indicating that subjects

remained in their original treatment group allotment, regardless of whether or not they

took their prescribed medication or, in the case of the control group, whether or not they remained medication free.

Any missing DXA or pQCT data points were filled by carrying the last available measurement values forward.

4.4.3.2 Changes from Baseline for Each Group at Each Time Point

Values for pQCT and DXA bone variables at each time point were compared to their baseline values for each treatment group using paired t-tests. Percent changes from baseline were also computed for pQCT bone variables at the 6, 12, 18 and 24-month time points for each subject while percent changes from baseline for DXA were calculated for the 12 and 24-month time points. Group means for percent change were then calculated from the values of the individual subjects. In certain situations, power and sample size calculations were also performed to determine the number of subjects needed to detect the observed changes as significant versus baseline.

4.4.3.3 Differences Between Groups

Repeated measures ANOVA was performed for each pQCT and DXA bone variable to determine whether significant differences between groups existed. Post-hoc testing of the repeated measures ANOVA was used to identify between which groups the differences existed. To determine differences between treatments at each time point, two-sample t-tests were used to compare two groups and one-way ANOVA was used to compare more than 2 groups. These tests were performed on the percent change from

baseline at each time point in order to compare these results to other trials (trabecular structure variables were considered as absolute changes from baseline). However, this statistical approach does not take into account that each time point is *not* independent of the other time points. One method that would take the repeated measures into account is comparing the area under the curve (AUC) for each treatment at each time point. The AUC calculations observe that each time point is dependent on the previous time point(s). T-tests and one-way ANOVA analyses were therefore also performed on the AUC for each variable to determine which treatment groups were different at each time point. Post-hoc testing was performed to determine which groups were different at each time point. Dunnett's t-tests (two-sided) were used to determine if any of the treatment regimens were significantly different than the control group at each of the time points. Tukey's (HSD) post-hoc test was used to identify any differences between any of the drug treatment groups at each time point. Significance was set at $p < 0.05$ for all tests unless otherwise specified. Dunnett's and Tukey's family error rates were set at 0.05.

Tukey's post-hoc test was chosen instead of Fisher's to compensate for multiple comparisons (i.e. between many different groups) within a single analysis. There were, however 27 different analyses performed, therefore, 1-2 comparisons could have been found to be statistically significant by chance alone at the $\alpha = 0.05$ level.

Some groups had lower baseline BMD than others, for example the '2-Tx' group had a significantly lower baseline BMD than the control group at the lumbar spine and lower than the '1-Tx' group at the femoral neck. Through calculations this difference may lead

to falsely elevated *percent* changes in the lower baseline BMD group, but not affect the

absolute changes. To adjust for these differences, an analysis of covariance (ANCOVA),

that adjusts for differences in baseline values could be employed, but was not used in the

statistical analysis of this thesis.

A note on trends in the data (i.e. p-value was close but not below 0.05): a trend in

the data could mean that the results (differences between groups or differences from

basline to a time point) are either a) *not true* or b) *true*, but not statistically significant due

to either too much noise/variation in the data or too small a sample size. This should be

kept in mind when assessing the value of data trends mentioned throughout this thesis.

CHAPTER 5: CLINICAL TRIAL BASELINE RESULTS AND DISCUSSION

5.1 Reproducibility of pQCT

5.1.1 Methods

5.1.1.1 *In Vitro* Reproducibility

The pQCT scanner is calibrated by the manufacturer for the conversion of attenuation values (cm^{-1}) into bone density values ($mg\ cm^{-3}$). *In vitro* measurements were performed using a polyethylene QA phantom ($0.495\ cm^{-1}$) to assess the long-term stability of the pQCT scanner. Phantom measurements were made every day that subjects were being tested during the study period of the prospective trial (3.5 years, May 1996 to November 1999).

5.1.1.2 *In Vivo* Reproducibility

A short-term reproducibility study using pQCT was performed on 23 individuals enrolled in the prospective trial. Two measurements were taken of the non-dominant arm in each subject as described in the methods chapter (section 4.3.3.1). After the first forearm scan was taken the subject removed their forearm completely from the measurement apparatus and then repositioned their arm for the second scan.

One method of characterizing the reproducibility of a measurement is to use precision errors. Precision error can be described by the standard deviation of the measurement (SD_{meas}). Frequently this estimate of precision is reported as a proportionate measure of SD_{meas} called the coefficient of variation (CV). The CV describes precision error as a percentage measure by dividing the SD_{meas} by the grand mean, then multiplying by 100 (mathematical equations found in FIGURE 5.1).

It has been noted, however, that the CV may underestimate the true imprecision of a measurement by up to 25%[Gluer et al. 1995]. Gluer et al. have therefore proposed the use of the root-mean-square CV (*rms*CV) which is calculated using the average root-mean-square SD_{meas} (*rms*SD_{meas}) (mathematical equations found in FIGURE 5.1)[Gluer et al. 1995]. The *rms*CV is also a proportionate estimate of precision and is therefore dependent on the mean of the given variable. Estimates of precision that are independent of the mean would be preferable and certainly more appropriate for variables that are distributed both above and below zero, such as the connectivity index. The bias and 95% limit of agreement for the differences observed between repeated measurements estimates precision using absolute values and has been used to describe short term precision[Sievanen et al. 1998]. This estimate of precision also gives information on the magnitude of change needed at an individual level to be confident that a real change has occurred. The 95% limit of agreement is from two SD below the average bias (difference) to two SD above the average bias (i.e. average bias ± 2 SD {SD_{meas}} of the difference) (mathematical equations found in FIGURE 5.1)[Bland and Altman 1986].

FIGURE 5.1

MATHEMATICAL MODELS FOR ESTIMATING PRECISION

$$SD_{meas} = \frac{\sum_{j=1}^{n} SD_j}{n} \quad , \quad CV = \frac{SD_{meas}}{\overline{X}} * 100$$

$$rmsSD_{meas} = \sqrt{\frac{\sum_{j=1}^{n} SD_j^2}{n}} \quad , \quad rmsCV = \frac{rmsSD_{meas}}{\overline{X}} * 100$$

$$\text{bias} \pm 95\% \text{ limit of agreement} = \frac{\sum_{j=1}^{n} x_{1j} - x_{2j}}{n} \pm 2SD_{meas}$$

Where: j is the subject index, n is the number of subjects, x_1 and x_2 are the first and second measurements in a single subject, $\overline{X}$ is the grand mean for the repeated measurements and SD_j is the standard deviation of a single subject (j) as follows:

$$SD_j = \sqrt{\frac{\sum_{i=1}^{m} \left(x_{ij} - \overline{x}_j\right)^2}{m-1}}$$

with m number of measurements,
i as the measurement index,
and x as the mean of the measurements
for that subject.

To simplify, for two measurements on each subject $\quad SD_j = \sqrt{\frac{d^2}{2}}$

The CV was calculated for all pQCT density, area and content measures as well as

*rms*CV and bias ± 95% limit of agreement for comparison. Precision estimates for all

structure measurements are reported as the average bias ± 95% limit of agreement and as

CV and *rms*CV for comparison, where applicable.

Two-sample t-tests were performed between the whole cohort (n=123) and the

reproducibility sample (n=23) to determine if there were any differences for age, height,

weight, DXA, or pQCT variables.

5.1.2   Results

5.1.2.1   *In Vitro* Reproducibility

Three hundred and eighty-three repeated measurements were performed using the

QA phantom over the extent of the prospective trial (3.5 years). This series of repeated

measurements showed that there was no machine drift during the study. The means and

standard deviations for density (mg cm^{-3}), attenuation (cm^{-1}) and voxels (#), as well as

precision estimates of the measurements are shown in TABLE 5.1.

TABLE 5.1

IN VITRO MEASUREMENTS OF QA PHANTOM: MEANS AND PRECISION

Bone Variable	Mean (SD)	Minimum	Maximum	CV
bone density (mg/cm^3)	263.0 (1.2)	259.3	265.4	0.4 %
attenuation (cm^{-1})	0.496 (0.001)	0.492	0.498	0.3 %
voxels (#)	1514 (3)	1506	1524	0.2 %

5.1.2.2 *In Vivo* Reproducibility

The descriptive statistics for the 23 subjects enrolled in the short-term

reproducibility study are shown in TABLE 5.2. Estimates of precision are reported as CV,

*rms*CV and bias ± 95% limit of agreement in TABLE 5.3. Of the nine pQCT variables,

total content was the most reproducible while trabecular content was the least

reproducible. Measurements of the total bone generally had the lowest precision error,

followed by cortical and finally trabecular compartments. For the total and cortical

compartments, bone content was more precise than volumetric bone density and cross-

sectional area was the least reproducible. Trabecular bone showed the opposite order with

cross-sectional area having the highest reproducibility followed by density and then

content.

This sample of 23 subjects was a representative sample of the whole cohort since no

differences between these two groups were found for any variable using two-sample t-

tests.

TABLE 5.2

DESCRIPTIVE STATISTICS FOR 23 SUBJECTS ENROLLED IN THE
SHORT-TERM *IN VIVO* REPRODUCIBILITY STUDY

Variable[a]	Mean (SD)	Minimum	Maximum
Age (years)	63.9 (8.8)	51.2	78.5
Height (cm)	158.1 (5.9)	143.0	168.0
Weight (kg)	63.0 (10.8)	42.0	86.0
LS T-score	-2.09 (1.55)	-4.83	3.57
FN T-score	-2.03 (0.70)	-3.21	-0.74
TOT-vBD (mg/cm^3)	344.1 (49.6)	270.9	434.0
TRAB-vBD (mg/cm^3)	179.1 (42.8)	113.5	286.3
CRT-vBD (mg/cm^3)	624.1 (78.9)	480.4	793.3
TOT-CSA (mm^2)	264.4 (40.4)	156.7	342.6
TRAB-CSA (mm^2)	167.9 (32.8)	81.1	236.4
CRT-CSA (mm^2)	96.6 (10.4)	74.1	118.4
TOT-CNT (mg)	225.0 (35.0)	167.5	324.1
TRAB-CNT (mg)	74.9 (24.1)	43.9	161.4
CRT-CNT (mg)	150.1 (21.1)	111.1	184.6
Ha (mm^2)	4.20 (2.98)	0.60	10.87
Hm (mm^2)	82.15 (41.99)	8.28	154.96
C.I.	-0.12 (9.32)	-14.23	17.40

[a] PQCT descriptive statistics are for two repeat measurements in 23 subjects

TABLE 5.3

ESTIMATES OF SHORT-TERM PQCT REPRODUCIBILITY IN 23 SUBJECTS

Variable	CV (%)	rmsCV (%)	Bias	± 95% Limit of Agreement
TOT-vBD (mg/cm^3)	2.2	2.8	-7.1	± 15.0
TRAB-vBD (mg/cm^3)	3.0	6.2	5.0	± 10.8
CRT-vBD (mg/cm^3)	4.0	5.2	-29.3	± 49.4
TOT-CSA (mm^2)	2.6	3.6	7.7	± 13.9
TRAB-CSA (mm^2)	3.7	5.3	4.0	± 12.5
CRT-CSA (mm^2)	3.9	5.5	3.8	± 7.6
TOT-CNT (mg)	1.4	2.0	2.5	± 6.2
TRAB-CNT (mg)	6.2	10.4	3.9	± 9.3
CRT-CNT (mg)	2.1	4.1	-1.4	± 6.2
Ha (mm^2)	10.8	17.3	-0.04	± 0.90
Hm (mm^2)	6.5	10.1	0.75	± 10.67
C.I.	N/A	N/A	0.24	± 2.69

5.2 Subjects

5.2.1 Number of Subjects: Recruited at Baseline and Included in Final Analyses

Of the 133 women who enrolled in the study, 123(92%) were included in the final

analyses. In order for a subject to be included in the final analyses, she had to have at

least 3 of the 5 scheduled pQCT scans performed and analyzed. Of the ten women who

were excluded from the analysis after having the initial scan, eight withdrew for personal

reasons (1-ALN + HRT, 1-ALN, 2-CNTL, 2-ETD + HRT, 1-ETD and 1-HRT), one

subject moved house and could not be contacted (ETD) and one subject's baseline pQCT

scan could not be analyzed (ETD + HRT). There were no trends for subjects to withdraw

from specific treatment groups. TABLE 5.4 shows the number of subjects recruited and the number of subjects analyzed for each treatment group.

TABLE 5.4

NUMBER OF SUBJECTS RECRUITED AND ANALYZED FOR
EACH OF THE SIX TREATMENT GROUPS

	ALN + HRT	ALN	CNTL	ETD + HRT	ETD	HRT	Total
Number of Subjects Recruited	10	25	34	13	29	22	**133**
Number of Subjects Analyzed	9	24	32	10	27	21	**123**

5.2.2 Subjects Who Were Disqualified but Still Followed

Six subjects were disqualified from the study but were still followed purely out of interest of the researchers and/or the patient. Reasons for disqualification were: one subject was not postmenopausal by FSH levels, one subject had broken both wrists in the past, one subject was diagnosed with cancer and was taking chemotherapy, one subject had a congenital bone disorder, and two subjects had been taking medications for osteoporosis prior to their appointment with the physician.

5.3   Initial Data Management

5.3.1   Retrospective Examination of Data

After all subjects had completed their 5th and final visit at the 2-year time point,

subject inclusion, pQCT and DXA data were inspected for errors. On retrospective

examination one subject in the control group did not meet the inclusion criteria for BMD

less than 1 SD below the young adult mean at the hip or spine. This error likely occurred

because the subject had an osteopenic DXA reading one year prior to study entrance and

this scan may have been confused with the scan performed at study entry. Data from this

patient were included in the analysis and inclusion in the trial was attributed to human

error. PQCT and DXA data were examined for outliers and these values were double-

checked for accurate entry into the spreadsheet. Three data entry errors (out of nearly

9000 entries) were detected and the numbers were corrected before analysis.

5.3.2   Missing Data Points

There was only one missing data point for all baseline measurements by DXA and

pQCT. One subject did not have a baseline lumbar spine scan performed. Data for DXA

lumbar spine are therefore describing a sample of 122 measurements.

5.3.3   Normality Testing

Tests for normality were performed on the baseline pQCT bone density data as a

whole cohort, after division into 2 groups ('Any-Tx' and control), after division into 3

groups ('1-Tx', '2-Tx' and control) and after splitting into all six treatment groups (5 active

therapy groups and 1 control group). Significance was set at p<0.05. When considering

the entire cohort, all baseline bone densities by pQCT (total, trabecular and cortical) were

found to be normally distributed. The pQCT bone density data was also normally

distributed for each of the individual groups when split into two, three and six separate

treatment groups.

5.4   Baseline Statistics

5.4.1   Baseline Descriptive Statistics

Baseline descriptive statistics were computed for age, height, weight, T-score for the

bone density measured by DXA at the femoral neck (FN) and lumbar spine (LS) and all

nine pQCT bone variables plus three trabecular structure indices. The descriptive

statistics for the total cohort are summarized in TABLE 5.5. The cohort was divided into

an 'Any-Tx' (one group including all subjects from the five different active therapy

groups together) or 'Control' group, with summary data presented in TABLE 5.6. The

cohort was also split into three groups: '1-Tx', '2-Tx' and control group. The baseline

statistics for these three groups are reported in TABLE 5.7. Finally, the cohort was

divided into six individual groups with five receiving active therapy and one control. The

baseline descriptive statistics for all six groups are shown in TABLE 5.8.

TABLE 5.5

BASELINE DESCRIPTIVE STATISTICS FOR TOTAL COHORT OF 123 SUBJECTS

Bone Variable	Mean (SD)	Minimum	Maximum
Age (years)	63.7 (9.0)	37.2	84.9
Height (cm)	158.7 (6.6)	142.0	178.0
Weight (kg)	64.5 (11.6)	41.0	110.0
LS T-score	-2.00 (1.28)*	-4.83	3.57
FN T-score	-2.15 (0.94)	-4.23	0.62
TOT-vBD (mg/cm^3)	350.4 (66.7)	197.1	531.0
TRAB-vBD (mg/cm^3)	174.1 (43.4)	89.2	288.1
CRT-vBD (mg/cm^3)	641.4 (101.2)	335.1	869.2
TOT-CSA (mm^2)	259.6 (38.3)	155.0	367.8
TRAB-CSA (mm^2)	163.7 (33.6)	82.0	258.1
CRT-CSA (mm^2)	95.8 (9.3)	73.0	121.4
TOT-CNT (mg)	223.5 (34.5)	141.4	324.0
TRAB-CNT (mg)	70.0 (19.1)	35.5	154.2
CRT-CNT (mg)	153.5 (27.6)	80.2	224.0
Ha (mm^2)	5.22 (5.60)	0.59	35.45
Hm (mm^2)	85.15 (44.16)	6.21	212.14
C.I.	-0.22 (8.66)	-15.30	18.16

* N=122 since one subject did not have a baseline lumbar spine DXA scan

TABLE 5.6

BASELINE DESCRIPTIVE STATISTICS BY TWO GROUPS:
'CONTROL' (N=32) versus 'ANY-TREATMENT' (N=91)

Bone Variable	Control (mean (SD))	'Any-Tx' (mean (SD))	T-test p-value
Age (years)	64.5 (9.4)	63.4 (8.9)	NS
Height (cm)	159.0 (6.4)	158.6 (6.7)	NS
Weight (kg)	68.3 (14.4)	63.1 (10.1)	NS
LS T-score	-1.57 (1.01)	-2.16 (1.33) *	0.012
FN T-score	-2.13 (1.09)	-2.15 (0.89)	NS
TOT-vBD (mg/cm^3)	358.9 (73.7)	347.4 (73.7)	NS
TRAB-vBD (mg/cm^3)	177.0 (41.6)	173.1 (41.6)	NS
CRT-vBD (mg/cm^3)	653.3 (102.9)	637.2 (102.9)	NS
TOT-CSA (mm^2)	263.1 (38.4)	258.3 (38.4)	NS
TRAB-CSA (mm^2)	165.2 (35.7)	163.2 (35.7)	NS
CRT-CSA (mm^2)	97.9 (8.5)	95.1 (8.5)	NS
TOT-CNT (mg)	231.2 (34.5)	220.8 (34.5)	NS
TRAB-CNT (mg)	71.4 (18.3)	69.5 (18.3)	NS
CRT-CNT (mg)	159.7 (28.6)	151.3 (28.6)	NS
Ha (mm^2)	4.18 (3.68)	5.58 (6.11)	NS
Hm (mm^2)	83.34 (45.31)	85.78 (43.98)	NS
C.I.	0.97 (8.75)	-0.64 (8.63)	NS

* N=90 since one subject did not have a baseline lumbar spine DXA scan

TABLE 5.7

BASELINE DESCRIPTIVE STATISTICS BY THREE GROUPS:
'1-Tx' (N=72), '2-Tx' (N=19) AND CONTROL (N=32)

Bone Variable	Control (mean (SD))	1-Tx (mean(SD))	2-Tx (mean(SD))	ANOVA p-value
Age (years)	64.5 (9.4)	63.5 (9.5)	63.3 (6.3)	NS
Height (cm)	159.0 (6.4)	158.8 (7.02)	158.0 (5.2)	NS
Weight (kg)	68.3 (14.4)[a]	64.3 (10.2)	58.5 (8.4)[a]	0.012[a]
LS T-score	-1.57 (1.01)[a]	-2.04 (1.41)	-2.65 (0.82)[a] *	0.015[a]
FN T-score	-2.13 (1.09)	-2.02 (0.86)[b]	-2.67 (0.80)[b]	0.026[b]
TOT-vBD (mg/cm^3)	358.9 (73.7)	349.5 (66.4)	339.6 (56.0)	NS
TRAB-vBD (mg/cm^3)	177.0 (41.6)	173.7 (46.6)	170.8 (34.5)	NS
CRT-vBD (mg/cm^3)	653.3 (102.9)	641.1 (101.4)	622.4 (100.1)	NS
TOT-CSA (mm^2)	263.1 (38.4)	258.2 (38.2)	259.0 (40.2)	NS
TRAB-CSA (mm^2)	165.2 (35.7)	163.1 (33.2)	163.7 (33.4)	NS
CRT-CSA (mm^2)	97.9 (8.5)	95.0 (9.3)	95.3 (10.3)	NS
TOT-CNT (mg)	231.2 (34.5)	221.8 (33.7)	217.0 (37.2)	NS
TRAB-CNT (mg)	71.4 (18.3)	69.7 (20.1)	68.8 (16.7)	NS
CRT-CNT (mg)	159.7 (28.6)	152.1 (26.7)	148.2 (28.3)	NS
Ha (mm^2)	4.18 (3.68)	5.70 (6.60)	5.11 (3.87)	NS
Hm (mm^2)	83.34 (45.31)	86.17 (44.19)	84.32 (44.33)	NS
C.I.	0.97 (8.75)	-0.53 (8.60)	-1.05 (8.98)	NS

* N=18 since one subject did not have a baseline lumbar spine DXA scan

[a] Post-hoc analysis determined the 2-Tx group was significantly different from control

[b] Post-hoc analysis determined the 2-Tx group was significantly different from 1-Tx

TABLE 5.8

BASELINE DESCRIPTIVE STATISTICS BY SIX TREATMENT GROUPS:
FIVE SEPARATE TREATMENT GROUPS AND ONE CONTROL GROUP

Bone Variable	ALN + HRT[#] (n=9)	ALN[#] (n=24)	CNTL[#] (n=32)	ETD + HRT[#] (n=10)	ETD[#] (n=27)	HRT[#] (n=21)	ANOVA p-value
Age (years)	62.7 (5.9)	62.4 (9.5)	64.5 (9.4)	63.8 (6.9)	68.8 (8.4)[a]	58.7 (8.2)[a]	0.011[a]
Height (cm)	158.7 (4.9)	159.4 (8.1)	159.0 (6.4)	157.4 (5.6)	156.6 (6.1)	160.9 (6.4)	NS
Weight (kg)	58.6 (9.8)	63.5 (7.4)	68.3 (14.4)	58.5 (7.6)	65.4 (12.7)	63.7 (9.9)	NS
LS T-score	-2.58 (0.72)	-2.12 (1.68)	-1.57 (1.01)	-2.72 (0.95) *	-2.19 (1.25)	-1.75 (1.28)	NS
FN T-score	-2.93 (0.77)	-2.19 (0.67)	-2.13 (1.09)	-2.43 (0.78)	-2.02 (1.05)	-1.82 (0.80)	NS
TOT-vBD (mg/cm^3)	317.5 (69.5)	351.8 (69.3)	358.9 (73.7)	359.4 (32.7)	344.6 (68.1)	353.3 (63.8)	NS
TRAB-vBD (mg/cm^3)	148.0 (26.1)	174.6 (55.1)	177.0 (41.6)	191.2 (28.1)	173.1 (44.8)	173.4 (40.2)	NS
CRT-vBD (mg/cm^3)	600.9 (130.8)	647.2 (108.1)	653.3 (102.9)	641.8 (62.9)	625.7 (107.4)	653.9 (86.7)	NS
TOT-CSA (mm^2)	262.1 (32.4)	257.4 (36.0)	263.1 (38.4)	256.2 (47.8)	253.7 (38.2)	264.7 (41.3)	NS
TRAB-CSA (mm^2)	166.2 (30.2)	162.4 (31.2)	165.2 (35.7)	161.3 (37.5)	159.8 (32.2)	168.1 (37.3)	NS
CRT-CSA (mm^2)	95.8 (7.2)	95.0 (11.2)	97.9 (8.5)	94.8 (12.8)	93.8 (8.1)	96.6 (8.7)	NS
TOT-CNT (mg)	204.8 (37.2)	224.0 (43.4)	231.2 (34.5)	227.9 (35.5)	214.5 (31.3)	228.7 (21.8)	NS
TRAB-CNT (mg)	60.7 (11.6)	70.7 (26.5)	71.4 (18.3)	76.1 (17.7)	68.1 (18.1)	70.5 (14.2)	NS
CRT-CNT (mg)	144.1 (34.4)	153.4 (28.7)	159.7 (28.6)	151.8 (22.7)	146.2 (24.8)	158.2 (26.6)	NS
Ha (mm^2)	7.08 (4.16)	7.09 (7.88)	4.18 (3.68)	3.34 (2.69)	5.78 (7.37)	4.02 (2.64)	NS
Hm (mm^2)	111.22 (36.50)	86.96 (43.92)	83.34 (45.31)	60.11 (37.04)	82.08 (41.87)	90.51 (48.90)	NS
C.I.	-6.84 (5.12)	-1.24 (9.51)	0.97 (8.75)	4.14 (8.48)	-0.34 (8.43)	0.048 (8.08)	NS

[#] Data reported as mean (SD)

[a] Post-hoc testing (Tukey's) showed that the HRT group was significantly different from the ETD group in age

* N=9 since one subject did not have a baseline lumbar spine DXA scan

5.4.2   Differences Between Groups at Baseline

To determine whether there were significant differences between groups at baseline, two-sample t-tests (two-tailed) were performed for each measurement when the cohort was divided into two groups (i.e. 'Any-Tx' versus control). One-way ANOVA was performed when the cohort was divided into more than two groups (i.e. divided into 3 groups: '1-Tx', '2-Tx' and control groups, or divided into 6 groups: 5 active therapy groups and 1 control group). Significant was set at $p<0.05$ and p-values are shown in the last column of each respective table.

When considering the two groups, 'Any-Tx' versus control, no significant differences were seen at baseline except for LS T-score by DXA. The control group had significantly higher mean T-score (-1.57 in control group versus –2.16 in the treatment group, $p<0.05$) at the lumbar spine.

When the cohort was divided into the '1-Tx', '2-Tx' and control groups significant differences were found with ANOVA at baseline for weight and both lumbar spine and femoral neck T-scores. Post-hoc analyses determined which groups differed. The '2-Tx' group was found to be lighter than the control group (58.5kg versus 68.3kg, respectively, $p=0.012$) and had a lumbar spine T-score that was lower than the control group (-2.65 versus –1.57, respectively, $P=0.015$) at baseline. The '2-Tx' group was also found to have a lower femoral neck T-score than the '1-Tx' group by 0.61 standard deviations. No other differences between these groups were found at baseline.

After division into the 6 separate treatment groups (5 active therapy and 1 control group), one-way ANOVA detected a significant difference in age ($p<0.05$) between groups. Post-hoc analysis determined that the HRT group was significantly different from the ETD group, with the ETD group being around 10 years older. No other differences between baseline values were found between the six groups.

5.4.3 Discriminating Between Osteopenic and Osteoporotic Groups

The objective of this analysis was to compare the abilities of DXA and pQCT bone variables to discriminate between osteopenic and osteoporotic classifications according to WHO definitions. Subjects with either or both of their LS and FN DXA T-scores equal to or lower than –2.5 were considered osteoporotic, while women whose DXA T-scores both fell between –1 and –2.5 were taken as osteopenic. Since the definitions of osteopenia and osteoporosis are based on DXA measurements, then this design inevitably means that DXA will appear to discriminate best between groups. DXA will therefore be used as a standard against which pQCT variables will be compared. One subject had DXA measurements on two different machines, giving different classifications. The values from the scan performed closest to the study entry date and therefore, closest to the pQCT measurement, was used for this analysis. There was one subject who was entered into the study due to evidence of vertebral fracture and who did not have either LS or FN T-scores below –1. Her data was not included in this analysis. The single subject whose DXA T-scores were both above –1, but was erroneously included in the prospective trial, was also not included in this analysis since the main objective involved classification into

osteopenic and osteoporotic groups. With the latter two subjects excluded, there were complete sets of data for 121 subjects included in this analysis. The difference between means of the two groups for all eleven measurements was expressed as the difference between groups divided by the weighted standard deviation (weighted according to the number of subjects in each group) of the two groups (z-statistic, shown in FIGURE 5.2). The higher the z-statistic, the better the measurement discriminates between the osteoporotic and osteopenic groups. Resulting z-statistic values for each bone variable are summarised in TABLE 5.9.

FIGURE 5.2

FORMULA FOR THE Z-STATISTIC

$$Z = \frac{mean\ 1 - mean\ 2}{\sqrt{\dfrac{S_1^2}{n_1} + \dfrac{S_2^2}{n_2}}}$$

TABLE 5.9

Z-STATISTIC VALUES* FOR DIFFERENT BONE VARIABLES DISCRIMINATING
BETWEEN OSTEOPENIA AND OSTEOPOROSIS

Variable	Z-Statistic
LS T-score	7.89^a
FN T-score	7.47^a
TOT-CNT	6.76^a
CRT-CNT	5.93^a
TOT-vBD	5.52^a
CRT-vBD	4.87^a
TRAB-vBD	4.30^a
Weight	4.16^a
Age$^{\#}$	3.83^a
C.I.	3.73^a
Ha$^{\#}$	3.17^b
Hm$^{\#}$	2.86^b
TRAB-CNT	2.82^b
CRT-CSA	2.51^c
Height	1.50^{NS}
TRAB-CSA$^{\#}$	1.23^{NS}
TOT-CSA$^{\#}$	0.48^{NS}

* Bone variables are listed in order of ability (best to worst) to discriminate between osteopenic and osteoporotic groups (i.e. highest to lowest z-statistic value).

$^{\#}$ These five variables had higher mean values in the osteoporotic group versus the osteopenic group, while all other variables had lower mean values in the osteoporotic group than the osteopenic group.

$^a = p< 0.001$ $^b = p<0.01$ $^c = p<0.05$ ^{NS} = not significant

5.5   Discussion

5.5.1   Reproducibility

The precision errors (CV) reported by the manufacturer for volumetric bone density

in the total, trabecular and cortical compartments are 5%, 3% and 9%, respectively using

the standard clinical software (version 5.21) in healthy individuals with good bone

mineral density[Norland Medical Systems Inc. 1999]. Other studies using the Stratec

XCT-900 or XCT-960 scanner and commercial software, generally reported lower

precision errors. CV values ranged from 0.8% to 1.2% for total density, 1.3% to 1.7% for

trabecular density and 0.9% to 1.9% for cortical density at the ultradistal radius[Butz et al.

1994; Martin, Campbell, and Reid 1999; Guglielmi et al. 2000]. None of these studies

reported the characteristics of the subjects involved. Boonen et al. (1997) measured *in*

vivo precision at the distal radius by pQCT in subjects from their study cohort, who were

healthy elderly women aged 70 to 87 years. In this population, they reported CV precision

errors that were slightly higher than the other three studies at 2.4%, 1.9% and 2.2% for

total, trabecular and cortical densities, respectively. This may suggest that different study

populations can introduce different amounts of variation in measurements and therefore

will affect *in vivo* reproducibility. In fact, Grampp et al. (1995) measured radial pQCT

reproducibility in healthy premenopausal and postmenopausal women as well as in

osteoporotic subjects and found that the precision error was higher in the osteoporotic

group than the premenopausal subjects for total and trabecular vBD and for cortical CNT.

In their study, the CV values in the postmenopausal osteoporotic group for total, trabecular and cortical vBD were 2.1%, 2.1% and 1.8% respectively and 5.6%for cortical CNT[Grampp et al. 1995].

In order to evaluate precision in the present study, repeat measurements at the ultradistal non-dominant radius were taken in 23 postmenopausal women aged 51-79 years with low bone density. Reported as CV for comparison, precision errors were found to be similar or slightly higher than those of the previously mentioned studies, with total, trabecular and cortical density CVs of 2.2%, 3.0% and 4.0%, respectively. Values of precision error as *rms*CV are 2.8%, 6.2% and 5.2% for TOT-vBD, TRAB-vBD and CRT-vBD, respectively. These *rms*CV values are similar or slightly higher than those reported for a previous *in vivo* precision study in 25 healthy volunteers using the same scanner and high-resolution software (xmice version 1.0) that found *rms*CV values for total, trabecular and cortical density at the distal radius of 2.7%, 4.9% and 3.7%, respectively[MacIntyre 1999]. Reasons for the present *in vivo* study having higher precision errors may include differences in the study population, acquisition software, scan line positioning and method for distinguishing between the cortical and trabecular compartments. The present study measured precision in postmenopausal women with low bone mass which means that, by definition, the CV will increase relative to subjects with higher bone density. Furthermore, the high-resolution acquisition software used in the present study takes longer to acquire the radial CT measurement than the standard Stratec pQCT software. The total scanning time of the scout view and measurement scan is 10

minutes using the high-resolution software and only approximately 6 minutes with the standard software. It becomes increasingly more difficult to stay motionless as the duration of measurement increases and some subjects in the present study did complain of pain or discomfort during the 10 minutes of scanning. The extended measurement time could lead to worse precision due to shifting and repositioning during the scan and movement has been shown to reduce precision[MacIntyre 1999]. The standard software also allows for automatic detection of the best position for the CT measurement in a repeat scan on the same patient, reducing repositioning error. This feature was not available on the high-resolution software version used in the present study, and therefore, could lead to higher precision errors. A further source of precision error could be the method of discriminating between cortical and trabecular bone. In the present study, an iterative contour detection method was used to determine the boundary between the trabecular bone core and the subcortical and cortical shell. Most pQCT studies use the Stratec standard mode that measures trabecular density in the inner 45% area of the total bone and determines cortical bone by an attenuation threshold. Differences in compartmentalization could conceivably affect precision.

5.5.2 Baseline Data

Baseline descriptive data for the entire cohort was similar to that reported for postmenopausal women from a previous study measuring a sample of subjects from the same region in Ontario, Canada and using the same pQCT scanner, high-resolution software and software analysis modes[MacIntyre, Adachi, and Webber 1999A].

When the total cohort was divided into the control and 'Any Treatment' groups,

there were no significant differences between groups except for lumbar spine T-score,

which was lower in the active therapy group than the control group (-2.16 and –1.57,

respectively, p<0.05). This should be expected since the decision to prescribe medication

was partially based on physician recommendation, and at the time of assessment, only

DXA measurements were available for evaluation. Furthermore, the diagnosis of

osteoporosis (according to the World Health Organization) is based on DXA

measurements at the hip and spine and the guidelines of the Canadian Medical

Association recommend the use of DXA for the assessment of osteoporotic fracture risk

in a clinical setting[Sturtridge, Lentle, and Hanley 1996]. The higher the fracture risk, the

more likely the physician would recommend treatment. What is surprising is that there

was no significant differences between the active therapy group and the control group for

femoral neck T-score, and in fact they were nearly identical (-2.15 and –2.13,

respectively, p=0.926). This suggests that the clinical decision to treat relied mostly on

lumbar spine T-scores or could indicate that spine differences are more common.

ANOVA testing of the differences at baseline between the '1-Tx', '2-Tx' and

control groups found differences in weight, lumbar spine T-score and femoral neck T-

score (p<0.05 for all). Lumbar spine T-score was again distributed among groups as

would be expected due to clinical decision making with the control group having the

highest mean LS T-score, the '2-Tx' group having the lowest LS T-score and the '1-Tx'

group being intermediate. Upon post-hoc testing, however, a significant difference was

only detected between the '2-Tx' and control groups. The '2-Tx' group had a LS T-score more than 1 SD lower than the control group, which would increase the risk for fracture by a factor of at least two[Marshall, Johnell, and Wedel 1996]. Femoral neck T-scores were found to be significantly lower in the '2-Tx' group than the '1-Tx' group (-2.67 and -2.02, respectively, p=0.026). Together these DXA data suggest that the clinical decision to prescribe any therapy was based mainly on the lumbar spine BMD, while the decision to prescribe combination therapy instead of only one therapy, may have been determined by lower femoral neck T-scores and their implications towards fracture risk. They also support that the women with the lowest bone densities were prescribed two therapies. The significant difference detected between groups in weight was found between the control group and the group of subjects taking two medications. The control group was nearly 10kg heavier than the '2-Tx' group. This can partially be explained by the positive correlation of weight to bone density[Hoover et al. 1996; Chen et al. 1997; Dargent-Molina, Poitiers, and Breart 2000], and also due to the projectional nature DXA measurement. Areal densities measured by DXA in subjects with bones of equal volumetric density would be higher in heavier subjects with bigger bones, and lower in lighter women with smaller bones[Genant et al. 1996]. Therefore, it is not surprising that the control group is slightly heavier and has higher bone density by DXA. When bone density and content was evaluated by pQCT, these volumetric measurements showed similar trends as the areal measurements. The control group had the highest content and density values, the '2-Tx' group had the lowest values and the '1-Tx' group was

intermediate for each of the total, trabecular and cortical compartments. These trends, however, did not reach significance between any of the three groups for any of the pQCT bone density or content variables.

After the cohort was divided into the control group and each of the five active therapy groups, ANOVA only detected significant differences between groups for age, while all other variables were similar between groups. Post-hoc testing determined that the ETD group was 10 years older than the HRT group. The probable reason for this difference is that younger women, who have more recently undergone menopause, are more inclined to go on hormone replacement therapy than older women[Phelan et al. 2001]. Hosking et al. found similar results in their trial since the group of women willing to potentially be randomized to HRT (or different alendronate doses) were less years since menopause than the women who chose to be assigned to different alendronate doses only, without HRT as a possibility[Hosking et al. 1998]. These findings are most likely due to the benefits of reducing menopausal symptoms as well as willingness in younger women to recommence menstruation if prescribed a cyclical regimen. Furthermore, the Ontario Drug Benefit Program covers etidronate therapy for osteoporosis for patients over 65 years of age[Government of Ontario 2000]. These health, convenience and financial reasons presumably led to older subjects preferring etidronate while younger women elected to go on hormone replacement therapy.

5.5.3 Discriminating Between Osteopenic and Osteoporotic Groups

The z-statistic evaluated how well the measurements of different bone characteristics were able to distinguish between a group of osteoporotic and osteopenic postmenopausal women, as defined by the WHO diagnostic criteria applied at the hip and spine. As would be expected, DXA measurements of the lumbar spine and femoral neck were the best at distinguishing between the two patient pools since these measurements were used as the original discriminating criteria. Even though measured trabecular bone, on average, accounts for around 60% of the bone area at the ultradistal radius, pQCT measurements of the total and cortical compartments both generally performed better than the equivalent variable measured in the trabecular compartment. Important to note, however, is that the total bone content in the 2.5mm slice at the ultradistal site is only approximately 30% trabecular bone and the other 70% is made up of cortical bone due to the vast difference in volumetric densities ($174mg/cm^3$ for trabecular and $641mg/cm^3$ for cortical bone). Other studies have found similar distributions of cross-sectional area (58-67% trabecular area) and bone content (27-34% trabecular content) for the trabecular and cortical compartments at the ultradistal radius[Gatti et al. 1996; MacIntyre, Adachi, and Webber 1999]. The trend that total radial measurements discriminate between groups better than either the cortical or trabecular compartments separately may reflect that DXA measurements, like the total compartment at the distal radius, are assessments of integral bone.

Another trend in pQCT measurements was that bone content variables were slightly better at discriminating between the two patient groups than volumetric bone mineral densities. This may be partially explained by both the DXA BMD measurements (and therefore T-scores) and the pQCT content measures being somewhat dependent on bone size while volumetric bone density is size independent. Since the determination of subject groups was based on DXA measurements, the trend for content to be better discriminators than density may be a reflection of the dependence of both DXA BMD and pQCT CNT on bone size. Trabecular content was an exception to the above trend and performed worse than total, cortical or trabecular volumetric densities. A study by Gatti and colleagues assessed changes in pQCT-measured bone variables at the distal radius with age[Gatti et al. 1996]. They found that total, cortical and trabecular volumetric densities and total and cortical contents, but not trabecular content, all decreased significantly with age. All of these same parameters seem to be better at discerning osteopenia from osteoporosis than trabecular content. Together, these data suggest that measuring trabecular content by pQCT at the ultradistal radius is not suitable for assessing increased fracture risk due to osteoporosis or for following skeletal changes over time.

The three indices of trabecular structure measured in this trial have previously been shown to change with age as would be expected[MacIntyre, Adachi, and Webber 1999A]. In a group of 145 subjects (88 women) 20 to 85 years old, Ha and Hm increased while C.I. decreased with age. Three other studies provide further evidence that these structural indices behave as expected in different situations at the distal radius. Ha has also been

shown to discriminate between non-fractured subjects and subjects with a recent wrist fracture, C.I. has been shown to be significantly correlated with bone strength *ex vivo* and Hm was seen to increase with unloading created by casting of the forearm[Gordon et al. 1998; MacIntyre 1999]. The z-statistic analyses also support that trabecular bone structure, as defined by Ha, Hm and C.I., follow the expected trends. For instance, Ha and Hm were both significantly larger (p=0.003 and p=0.005, respectively) in the osteoporotic group, while the connectivity index was significantly lower (p<0.001) in the osteoporotic subjects. These data also suggest that the structural indices are better than geometric pQCT measurements in discriminating between groups. Whereas all three structure variables were able to significantly discriminate between the two groups, of the cross-sectional area measurements, only cortical area could detect significant differences, while total and trabecular area could not. Cortical cross-sectional area was significantly smaller in the osteoporotic group while trabecular and total areas showed a trend to be larger in the osteoporotic group than the osteopenic group. These trends in geometry for each of the three separate bone compartments are supported by the findings from other trials evaluating changes at the distal radius with age. Studies using pQCT *in vivo* have been able to detect increases in total and trabecular cross-sectional area with age in the distal radius and some have also found that the cortical area decreases with age[Gatti et al. 1996; Wapniarz et al. 1997; Nijs et al. 1998]. This shows that expansion in the trabecular core occurs at the expense of the cortical shell, suggesting that bone is resorbed at the endocortical surface, also known as the process of cortical trabecularization. Since the

cortex thins with age, the endocortical resorption must be proceeding at a rate faster than the periosteal bone apposition. The enlargement of the total bone cross-section is thought to be a structural compensation to help maintain bone strength as bone mass diminishes with age. Redistribution of the same amount of bone mass outwards should not alter compressive strength, but will have the advantage of increasing the moment of inertia and therefore will improve bending strength[Bouxsein, Myers, and Hayes 1996].

For comparison, age, height and weight were also tested with the z-statistic. Significant differences in age and weight were seen between the osteopenic and osteoporotic groups and these characteristics suggested a slightly better discriminatory ability than the structure indices and measures of radial cross-sectional geometry, but worse than the pQCT measures of density and content at the ultradistal radius. Height was not found to differ significantly between the two groups, but there was a trend for the osteoporotic women to be slightly shorter.

A limitation of this study is that the division of subjects into osteopenic and osteoporotic groups was based on DXA measurements. A less biased approach may be to evaluate the ability of different bone variables to discriminate between fracture (defining high-risk individuals) and non-fracture (indicating normal or lower risk individuals). Grampp et al. (1995) evaluated the ability of DXA and pQCT measurements to distinguish between postmenopausal women who were either considered osteoporotic (≥ 1 vertebral fracture) or controls[Grampp et al. 1995]. Using pQCT at the distal radius, they found that cortical content and area were able to significantly distinguish between the

osteoporotic and control group (p≤0.005), and that total density and content showed a

trend to be able to distinguish between the two groups (p=0.052 and p=0.069,

respectively). The present study also observed that cortical area could significantly

distinguish between high and low-risk groups, but total measures of content and density

were found to be better at discriminating between the two groups than cortical area.

Inconsistencies between the present study and the study by Grampp et al. could be due to

differences in the definition of osteoporosis and differences in the method for separation

of the trabecular and cortical compartments. Although some findings varied, these two

studies both suggest that total and cortical measurements discriminate between high

fracture risk and low fracture risk groups better than trabecular

CHAPTER 6: CLINICAL PROSPECTIVE TRIAL RESULTS AND DISCUSSION

6.1 Initial Data Management

Retrospective examination of data for spreadsheet entry errors and normality testing was performed as outlined in chapter five.

6.1.1 Missing Data Points

There were four DXA data points missing. One baseline lumbar spine scan was not performed on a single subject. One subject missed her 12-month DXA measurements and two subjects missed their 24-month DXA scans. Data from the last point of measurement were carried forward to fill these missing values. The lumbar spine data for the subject who did not have a baseline measurement was excluded from analyses.

All subjects had baseline pQCT scans. Three subjects missed their 6-month appointment, two missed the 12-month measurement, two missed the 18-month scan and data from seven subjects are missing at the 24-month time point. As well, two subjects in the control group broke their non-dominant wrist, which was the forearm of measurement in both cases. In one subject, this occurred just prior to the 18-month visit and therefore the 18 and 24-month pQCT scans for this patient were excluded from the analysis. In the second subject, the fracture occurred between the 18 and 24-month

measurements, causing her 24-month scan to be excluded. The total number of pQCT

scans that were either missing or excluded from the analysis was seventeen. This means

that 97% (598 out of a possible total of 615) of the pQCT scans were included in the final

analysis. Table 6.1 shows the distribution of included scans by treatment group. There

was no trend for data to be missing from one treatment group more than any other group.

As with DXA, data from the last point of measurement were carried forward for these

missing or excluded scans.

There were 19 missing data points for the trabecular structure analysis of pQCT

images at the distal radius, which was similar to the pQCT density data points.

Differences in missing data points between density and structure data were due to the

following reasons: two of the images were incomplete when downloaded to floppy disk

for analysis on the Unix system, one image could not be analyzed properly by the

structure software and one pQCT scan which was missing bone density data could still be

transferred and analyzed for trabecular structure indices. Data from the last point of

measurement were carried forward to fill the missing values.

TABLE 6.1

NUMBER OF PQCT DENSITY MEASUREMENTS INCLUDED IN THE ANALYSIS
AT EACH TIME POINT BY TREATMENT GROUP

Treatment	Baseline	6 Months	12 Months	18 Months	24 Months
ALN + HRT	9	9	9	9	9
ALN	24	23	24	23	23
CNTL	32	32	31	31[a]	29[b]
ETD + HRT	10	10	10	10	10
ETD	27	25	26	27	24
HRT	21	21	21	20	20

[a] One subject had fractured their measured wrist and data was excluded from analysis
[b] Two subjects had fractured their measured wrists and data was excluded from analysis

6.2   DXA: Bone Mineral Density

6.2.1   DXA: Changes from Baseline

The mean percent changes from baseline for 12 and 24 months at the lumbar spine

and the femoral neck were calculated for all groups after three distinct divisions of the

total cohort into treatment regimens. First the cohort was divided into two groups, those

subjects taking any of the prescribed medications for osteoporosis (named the 'Any

Treatment' or 'Any-Tx' group) and those not taking any osteoporosis medications

(Control group). Analyses were re-run after the second division of the cohort into three

groups. The three groups were those subjects taking 1 drug treatment (1-Tx), subjects

taking 2 different anti-resorptive drugs (2-Tx) or subjects taking no medication for

osteoporosis (Control). Finally, the cohort was divided into the six separate treatment

groups (A+H, ALN, E+H, ETD, HRT and CNTL groups) and the data was re-analyzed.

To determine whether each group gained or lost a statistically significant amount of BMD

at either DXA site, paired t-tests were performed. Paired t-tests compared baseline to 12-

month values and baseline to 24-month values separately for the femoral neck and the

lumbar spine (see TABLES 6.2-6.4).

6.2.1.1   Cohort Divided into 2 Groups: 'Any Treatment' and Control

After division of the cohort into the two groups ('Any-Tx' and 'Control'), the

control group (n=32) was found to have lost a small amount of bone at the spine and hip

(0.5% to 1.2%), but these losses did not reach statistical significance at either 12 months

or 24 months for the hip or the spine. Conversely, the group of 91 subjects who were

taking any active drug therapy had significant gains in BMD at both the 12 and 24-month

marks for the spine (3.8% and 5.5% gains, p<0.001) and the hip (1.5% and 1.6% gains,

p<0.05) (see TABLE 6.2).

TABLE 6.2

MEAN PERCENT CHANGES FROM BASELINE AT 12 AND 24 MONTHS FOR DXA BMD OF THE LUMBAR SPINE AND FEMORAL NECK FOR 'ANY TREATMENT' AND 'CONTROL' GROUPS

Treatment Group	LS 12 Months		LS 24 Months		FN 12 Months		FN 24 Months	
	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
Control (n=32)	-0.7 (3.0)	0.138	-1.2 (3.4)	0.062	-0.5 (5.6)	0.518	-0.6 (5.9)	0.591
Any Treatment (n=91)	3.8 (3.8)*	0.000	5.5 (5.6)*	0.000	1.5 (5.3)	0.033	1.6 (5.7)	0.013

* The means for these groups were missing one scan (i.e. n=90)
LS – lumbar spine, FN – femoral neck

6.2.1.2   Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

When considering the 1-Tx and 2-Tx groups separately, the subjects on only one

drug therapy had significant mean gains at the lumbar spine at 12-months of 3.4%

($p<0.001$) and at 24-months of 4.7% ($p<0.001$). This group did not, however, gain BMD

at the femoral neck at either 12 or 24-months (0.8% and 0.4%, $p=0.36$ and 0.59

respectively). The group of subjects taking two medications had highly significant gains

at both the spine and the hip at both time points. Lumbar spine BMD gains were 5.5%

($p<0.001$) and 8.6% ($p<0.001$) at 12 and 24 months, respectively. Femoral neck BMD

gains were 4.2% ($p<0.005$) and 6.4% ($p<0.001$) at 12 and 24 months, respectively for the

'2-Tx' group (see TABLE 6.3).

TABLE 6.3

MEAN PERCENT CHANGES FROM BASELINE AT 12 AND 24 MONTHS FOR DXA BMD OF THE LUMBAR SPINE
AND FEMORAL NECK FOR '1-Tx', '2-Tx' AND 'CONTROL' GROUPS

Treatment Group	LS 12 Months		LS 24 Months		FN 12 Months		FN 24 Months	
	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
Control (n=32)	-0.7 (3.0)	0.138	-1.2 (3.4)	0.062	-0.5 (5.6)	0.518	-0.6 (5.9)	0.591
1-Tx (n=72)	3.4 (3.7)	0.000	4.7 (4.3)	0.000	0.8 (5.1)	0.365	0.4 (4.7)	0.590
2-Tx (n=19)	5.5 (4.1)*	0.000	8.6 (4.5)*	0.000	4.2 (5.1)	0.003	6.4 (6.4)	0.000

* The means for these groups were missing one scan (i.e. n=18)
LS – lumbar spine, FN – femoral neck

6.2.1.3   Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

Once the cohort was divided into the 5 separate active therapy groups and the control group, analyses determined that each of the 5 individual treatment regimens produced highly significant gains in LS BMD at both the 12 and 24-month marks(see TABLE 6.4). Changes in spinal BMD from baseline at 12 months ranged from 3.1% in the ETD group to 5.6% in the E+H group (p-values ranged from p<0.001 to 0.012). At 24 months, BMD gains at the lumbar spine ranged from 4.0% in the ETD group to 9.3% in the A+H group ($p \leq 0.001$ for all 5 treatment groups). Although the control group lost 0.7% at 12 months and 1.2% by the 24-month mark, these changes in LS BMD were not significant (p=0.138 and 0.062, respectively). At the femoral neck, significant changes in BMD from baseline values were only detected in two of the treatment groups. The ALN group gained 2.0% by 12 months (p<0.05), but by 24 months the gain over baseline declined to 1.7% and was no longer significant (p=0.066). The A+H group gained significant BMD at the femoral neck by both 12 and 24-month marks. At 12 months the A+H group had gained 6.4% (p=0.001) and by 24 months the gain had increased to 8.4% (p=0.001) over baseline values. By 24 months, the E+H group showed a trend for BMD gains with a 4.5% increase, but this was not significant relative to baseline (p=0.072). The ETD group showed a weak trend for a loss in FN BMD of 0.9% from baseline at 24 months (p=0.226). Femoral neck BMD did not significantly change over the trial in the control group.

TABLE 6.4

MEAN PERCENT CHANGES FROM BASELINE AT 12 AND 24 MONTHS FOR DXA BMD OF THE LUMBAR SPINE
AND FEMORAL NECK FOR ALL SIX TREATMENT GROUPS

Treatment Group	LS 12 Months		LS 24 Months		FN 12 Months		FN 24 Months	
	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
CNTL (n=32)	-0.7 (3.0)	0.138	-1.2 (3.4)	0.062	-0.5 (5.6)	0.518	-0.6 (5.9)	0.591
A + H (n=9)	5.4 (3.7)	0.002	9.3 (4.8)	0.001	6.4 (4.0)	0.001	8.4 (5.2)	0.001
ALN (n=24)	3.9 (3.0)	0.000	5.7 (3.2)	0.000	2.0 (3.9)	0.012	1.7 (4.6)	0.066
E + H (n=10)	5.6 (4.8)*	0.012	8.0 (4.4)*	0.001	2.2 (5.4)	0.268	4.5 (7.1)	0.072
ETD (n=27)	3.1 (3.4)	0.000	4.0 (4.1)	0.000	0.5 (5.9)	0.913	-0.9 (4.4)	0.226
HRT (n=21)	3.2 (4.8)	0.006	4.4 (5.4)	0.001	-0.2 (5.3)	0.679	0.5 (5.0)	0.747

* The means for these groups were missing one scan (i.e. n=9)
LS – lumbar spine, FN – femoral neck

6.2.2   DXA: Comparison of Different Treatment Regimens

Repeated measures ANOVAs were used to examine if any differences existed

between treatments for LS BMD and FN BMD over the 2-year trial. Post-hoc tests for

repeated measures ANOVA were used to determine between which treatments the

differences existed. In order to check at which time point differences between treatments

existed, one-way ANOVA was used for both the percent change from baseline and the

area under the curve (AUC) from baseline. Post-hoc tests for one-way ANOVA were

used to identify which treatments were different at each time point. See section 4.4.3.3 for

further details.

6.2.2.1   Cohort Divided into 2 Groups: 'Any Treatment' and Control

Repeated measures ANOVA found significant differences between subjects taking

anti-resorptive therapy and those who were not on any treatment at both the lumbar spine

($p<0.001$) and the femoral neck ($p<0.05$) over the two-year trial. T-tests on percent

changes from baseline and AUC from baseline gave very similar results at the 12-month

and 24-month time points for both the spine and the hip. For the spine, these tests showed

that the 'Any Treatment' group gained significantly more bone than the control group at

both the 12 and 24-month time points ($p<0.001$ for both). At the hip, however, both

statistical methods showed no significant difference between groups at either 12 or 24-

months, although there was an obvious trend that the subjects taking treatment gained

more BMD than the control subjects (p-values: 0.060 to 0.077) (see FIGURE 6.1).

FIGURE 6.1

DXA PERCENT CHANGE OVER BASELINE AT 12 AND 24 MONTHS FOR
THE CONTROL AND 'ANY-TREATMENT' GROUPS AT THE
A) LUMBAR SPINE and B) FEMORAL NECK

A)

**LS DXA Percent Change from Baseline
for Any-Tx and Control Groups**

B)

**FN DXA Percent Change from Baseline
for Any-Tx and Control Groups**
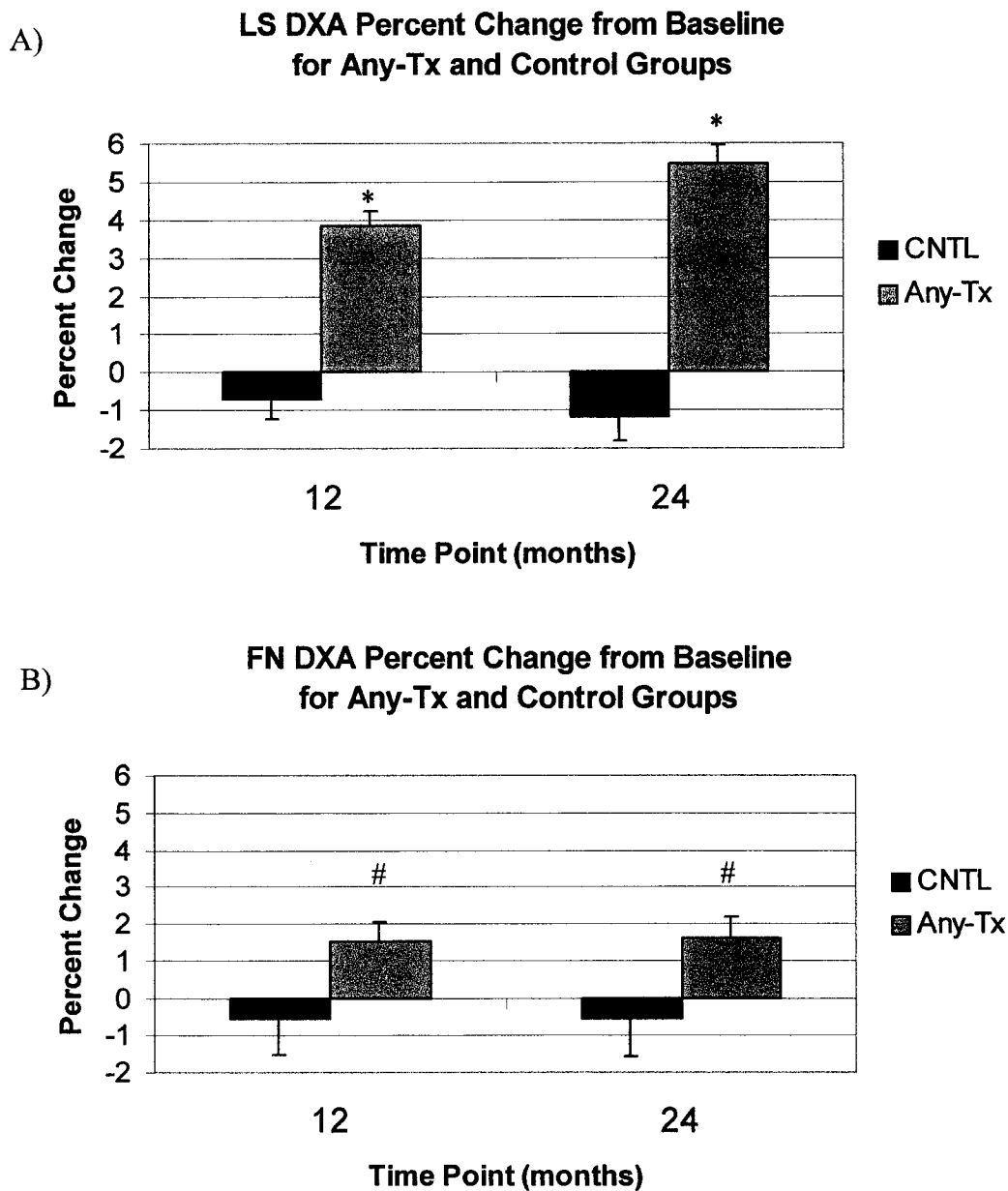
* Significantly different from Control group, p<0.001
# AUC T-test of Any-Tx versus CNTL: p=0.077 at 12 months and p=0.060 at 24 months

6.2.2.2   Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

When considering the treatment regimens as '1-Tx', '2-Tx' and 'Control' repeated

measures ANOVA found significant differences at both the lumbar spine ($p<0.001$) and

femoral neck ($p<0.001$) for the 2-year trial. At the lumbar spine, post-hoc tests on the

repeated measures ANOVA revealed that all three treatment groups were significantly

different from each other over the two years. The '2-Tx' group gained more LS BMD

than the '1-Tx' or control groups and the '1-Tx' group gained more bone density at the

spine than the control group over the two-year trial. One-way ANOVA tests on percent

change and AUC at the spine gave similar results at each time point and showed that

there were differences between groups at both the 12-month and 24-month time points.

Post-hoc analysis showed that both the '1-Tx' and '2-Tx' groups gained significantly

more BMD at the spine than the control group at the 12-month and 24-month marks. At

the 24-month time point, the '2-Tx' group was also found to have gained more spinal

BMD than the '1-Tx' group.

At the femoral neck, repeated measures ANOVA post-hoc testing revealed that over

the two-year trial, the '2-Tx' group was significantly different than the '1-Tx' group and

the control group, but there was no significant difference between the '1-Tx' and control

groups. Again, the two methods used in one-way ANOVA found very similar results,

with differences between groups being detected at both the 12-month and 24-month time

points. Post-hoc testing determined that at both time points, the '2-Tx' group had gained

significantly more bone than either the '1-Tx' group or the 'Control' group (see FIGURE

6.2). Although the '1-Tx' group gained around 1% and the control group lost

approximately 1% by 24 months, the difference between these two groups was not

significant (Dunnett's t-test: CI_{95} -0.58 to 1.92).

FIGURE 6.2

DXA PERCENT CHANGE OVER BASELINE AT 12 AND 24 MONTHS FOR
THE CONTROL, '1-TX' AND '2-TX' GROUPS AT THE
A) LUMBAR SPINE and B) FEMORAL NECK

A)

**LS DXA Percent Change from Baseline
for 1-Tx, 2-Tx and Control Groups**

B)

**FN DXA Percent Change from Baseline
for 1-Tx, 2-Tx and Control Groups**
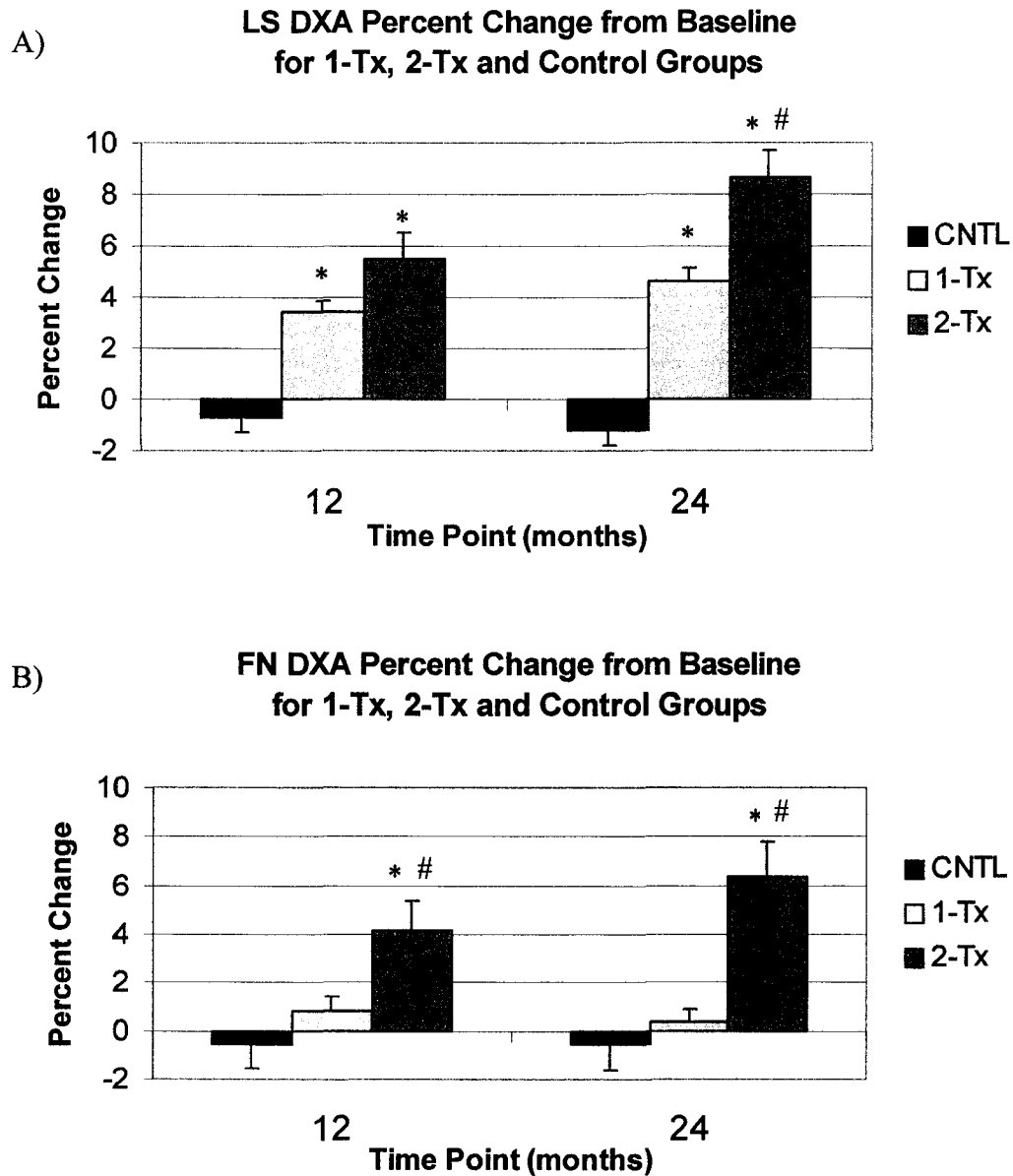
* Significantly different from Control group, $p<0.05$ (Dunnett's post-hoc)
# Significantly different from '1-Tx' group, $p<0.05$ (Tukey's post-hoc)

6.2.2.3 Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

After division of the total cohort into the six individual treatment groups, repeated

measures ANOVA detected significant differences between treatment regimens over the

two-year trial at the lumbar spine and the femoral neck. At the lumbar spine, post-hoc

testing revealed that each of the individual treatment groups was significantly different

from the control group, and the A+H group was borderline significantly different from

the ETD group (p=0.051). When considering the 12-month and 24-month marks

separately with one-way ANOVA, significant differences were found for the spine at

each time point. Post-hoc testing showed that all five active treatment groups gained

significantly more spinal bone by 12 and 24 months than the control group. Using percent

change from baseline also showed that the A+H group gained significantly more bone

than the ETD or HRT groups at 24-months (see FIGURE 6.3), but this was not detected

when using the AUC values.

At the femoral neck, repeated measures ANOVA detected greater BMD gains in the

A+H group than any of the three single therapy groups (ALN, HRT and ETD) or the

control group over the two-year trial. One-way ANOVA of AUC showed significant

differences between groups at both the 12-month and 24-month time points. Post-hoc

testing revealed that the A+H group had gained more BMD at the hip than either the

control, ETD or HRT groups at both the 12 and 24-month marks. Using percent change

from baseline at 24-months also showed that the A+H group gained significantly more

bone than the ALN group, and that the E+H group had gained significantly more bone

than the control group (see FIGURE 6.4). These differences were not detected using the

AUC method.

FIGURE 6.3

DXA PERCENT CHANGE OVER BASELINE AT 12 AND 24 MONTHS FOR CONTROL AND 5 DIFFERENT TREATMENT GROUPS AT THE LUMBAR SPINE
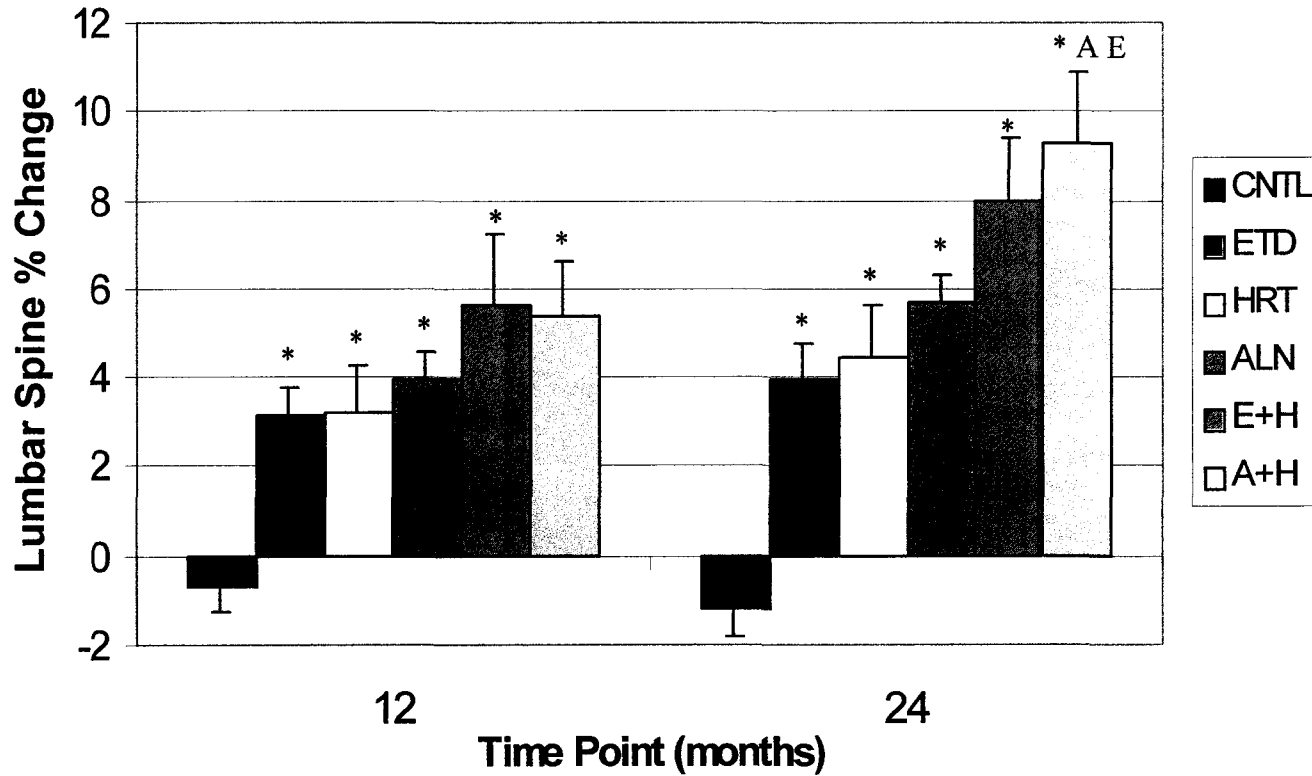
* Significantly different from Control group, p<0.05 (Dunnett's post-hoc)

A Significantly different from ALN; E Significantly different from ETD; p<0.05 for both (Tukey's post-hoc)

FIGURE 6.4

DXA PERCENT CHANGE OVER BASELINE AT 12 AND 24 MONTHS FOR CONTROL AND 5 DIFFERENT TREATMENT GROUPS AT THE FEMORAL NECK
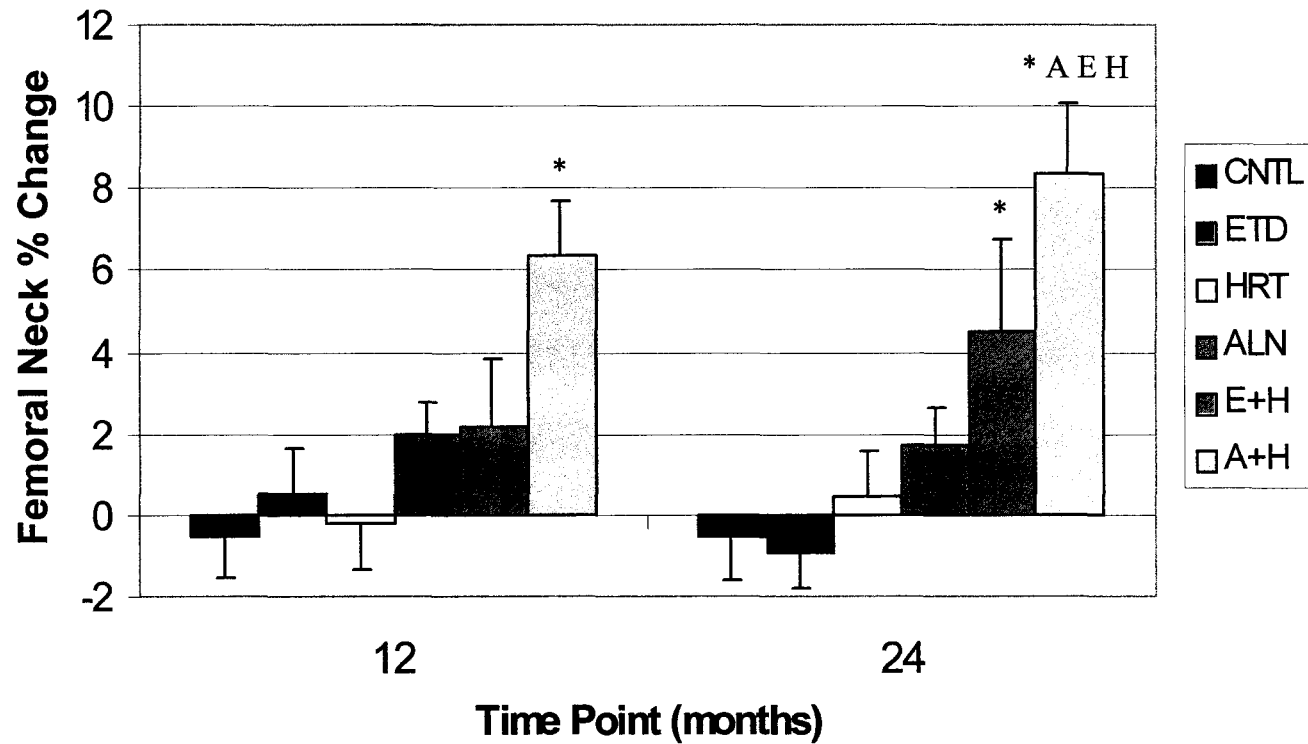
* Significantly different from Control group, p<0.05 (Dunnett's post-hoc)
A Significantly different from ALN; E Significantly different from ETD;
H Significantly different from HRT; p<0.05 for all three (Tukey's post-hoc)

6.3   PQCT: Bone Density, Content and Cross-sectional Area

6.3.1   PQCT: Changes from Baseline

The total cohort was divided into treatment regimens by three different sets of

criteria as described for DXA (see section 6.2.1). Percent changes from baseline to each

of 6, 12, 18, 24 months were computed for each subject, and the mean percent changes

were calculated for each of the different treatment regimens (see TABLES 6.5-6.9).

Paired t-tests for each pQCT bone variable compared absolute values at each time point

(6, 12, 18 and 24-months) to corresponding baseline values to determine if significant

changes had occurred.

6.3.1.1   Cohort Divided into 2 Groups: 'Any Treatment' and Control

The control group lost nearly 2% TOT-vBD by the 24-month mark (p=0.052). The

trabecular compartment showed a trend at every time point for a loss in trabecular density

(-1.3, p=0.157 at 2 years). Cortical density, on the other hand, did not show a clear trend

towards an increase or decrease (-0.7% to 0.8% changes from baseline) and no significant

differences were seen from baseline at any time point (see TABLE 6.5). Considering

bone content measures, the control group showed significant losses (1.3 to 2.2%, p-value

= 0.000 to 0.019) from baseline at all time points for TOT-CNT. Cortical content losses

were also significant from baseline values at 12-months and 24-months in the control

group (2.1% and 2.3% losses respectively, p<0.05 for both). Trabecular content remained

stable. No other statistically significant changes were seen from baseline for the control

group, although trends suggest that the TRAB-CSA enlarged and CRT-CSA decreased while TOT-CSA showed no change from baseline at two years.

The 'Any Treatment' group had significant changes from baseline at nearly all time points for all nine pQCT variables except CRT-CSA which only differed significantly from baseline values at the 18-month mark (1.2% loss, $p<0.05$) (see TABLE 6.5). In general, the 'Any-Tx' group gained total and cortical volumetric bone density while losing trabecular bone density. Total and trabecular cross-sectional areas decreased along with small decreases in total bone content (0.5-1.1%) and larger decreases in trabecular bone content (3.7-5.0%). Cortical content, however, increased over the two years, with significant increases at 12, 18, and 24 months (1.5-1.7% gains, p-values 0.008-0.022).

TABLE 6.5

MEAN PERCENT CHANGES FROM BASELINE FOR PQCT RADIAL BONE VARIABLES AT EACH TIME POINT
FOR 'ANY TREATMENT' AND 'CONTROL' GROUPS

Treatment Group	Bone Variable	6 Months		12 Months		18 Months		24 Months	
		Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
Control (n=32)	TOT-vBD	-0.4 (3.7)	0.530	-0.9 (4.5)	0.209	-0.8 (5.0)	0.294	-1.9 (5.7)	0.052
	TRAB-vBD	-0.5 (8.4)	0.522	-1.2 (6.5)	0.111	-0.6 (6.6)	0.263	-1.3 (7.8)	0.157
	CRT-vBD	0.0 (6.0)	0.939	0.8 (6.7)	0.538	-0.1 (6.6)	0.871	-0.7 (7.9)	0.514
	TOT-CSA	-0.8 (3.9)	0.329	-1.2 (4.0)	0.146	-0.4 (4.7)	0.610	0.1 (5.2)	0.769
	TRAB-CSA	-0.4 (7.1)	0.664	0.1 (7.1)	0.940	0.6 (7.9)	0.852	1.6 (8.5)	0.377
	CRT-CSA	-0.7 (6.7)	0.427	-2.5 (7.5)	0.068	-1.3 (6.7)	0.213	-1.3 (8.0)	0.326
	TOT-CNT	-1.3 (2.3)	0.002	-2.2 (3.2)	0.000	-1.4 (3.6)	0.019	-2.0 (4.0)	0.005
	TRAB-CNT	-0.7 (13.6)	0.429	-1.0 (10.4)	0.240	0.2 (12.0)	0.690	0.6 (15.3)	0.797
	CRT-CNT	-0.9 (4.9)	0.237	-2.1 (5.1)	0.015	-1.6 (5.9)	0.079	-2.3 (6.5)	0.029
Any Treatment (n=91)	TOT-vBD	1.0 (4.8)	0.075	1.2 (5.4)	0.042	1.5 (8.1)	0.022	1.3 (5.8)	0.074
	TRAB-vBD	-1.9 (7.5)	0.004	-2.3 (7.8)	0.001	-3.0 (8.1)	0.000	-2.9 (7.4)	0.000
	CRT-vBD	2.0 (7.9)	0.018	2.3 (9.7)	0.038	3.2 (6.9)	0.000	2.8 (8.5)	0.005
	TOT-CSA	-1.7 (5.2)	0.001	-1.6 (5.6)	0.008	-2.4 (7.7)	0.002	-1.5 (5.6)	0.007
	TRAB-CSA	-2.0 (7.0)	0.004	-2.1 (7.4)	0.005	-2.3 (5.7)	0.005	-1.9 (7.2)	0.011
	CRT-CSA	-0.8 (7.2)	0.163	-0.2 (7.7)	0.598	-1.2 (5.4)	0.017	-0.7 (6.7)	0.165
	TOT-CNT	-0.9 (3.6)	0.025	-0.6 (3.1)	0.055	-1.1 (12.8)	0.034	-0.5 (4.0)	0.156
	TRAB-CNT	-3.7 (11.1)	0.000	-4.2 (12.6)	0.000	-5.0 (5.5)	0.000	-4.6 (11.4)	0.000
	CRT-CNT	0.8 (5.3)	0.141	1.5 (5.0)	0.008	1.6 (5.8)	0.013	1.7 (5.7)	0.022

6.3.1.2   Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

Changes from baseline for the control group have already been reported above

(section 6.3.1.1, also see TABLE 6.5).

The group of subjects taking only one anti-resorptive therapy ('1-Tx', n=72) had a

relatively constant total bone density at the distal radius. Within the separate

compartments, however, there were significant trabecular bone density losses at all time

points (2.2-3.6%, $p<0.001$ to $p=0.003$) while cortical bone density increased significantly

over baseline values at 18 and 24 months (2.8% and 2.4 respectively, both $p<0.05$) (see

TABLE 6.6). Bone area significantly decreased for total (1.5-2.5% losses, p-values 0.006

to 0.033) and trabecular (2.0-2.3% losses, p-values 0.008 to 0.023) compartments at all

time points, but cortical bone area was only significantly lower than baseline at the 18-

month mark (1.3% loss, $p<0.05$). Total bone content decreases relative to baseline values

by 0.9-1.8%, which was mainly due to losses in trabecular bone content which declined

4.2-5.3% ($p<0.001$ for all time points). Cortical bone content increased slightly over

baseline (1.4% at 24 months, $p=0.092$), but these increases did not reach significance.

The '2-Tx' group of subjects who were taking two anti-resorptive therapies (either

A+H or E+H, n=19) had significant increases in total radial bone density at 6, 12 and 18

months (2.5-3.5% gains, p-values 0.004-0.010). Although there was a 2.5% gain in total

bone content over baseline at 24 months, this did not reach significance ($p=0.107$). The

gains in total bone density were likely due to cortical bone since the cortex significantly

increased in density over baseline at all time points by 4.2-5.5% (p-values 0.005 to

0.049). Trabecular density, however, showed a decreasing trend (0.7-1.7%), but was not significantly lower than baseline at any time point. There were also decreasing trends for cross-sectional areas in all compartments (total area decreased 1.3-1.8%, trabecular 1.2-2.2% and cortical 0.9-2.3%), but these losses were only significant for total (1.8% loss, $p < 0.05$) and trabecular (2.2% loss, $p < 0.05$) bone compartments at 12 months. In general, total bone content remained stable or tended to increase slightly (0.5-1.5% gains, p-values not significant) while bone was lost in the trabecular compartment (2.0-3.8% losses) and gained in the cortical compartment (2.6-3.6% gains). The increases in cortical content were significant at 6, 12 and 18 months ($p \leq 0.001$ all time points), but decreases in trabecular content was only found to be significantly lower than baseline at 12 months (3.8% loss, $p < 0.05$).

TABLE 6.6

MEAN PERCENT CHANGES FROM BASELINE FOR PQCT RADIAL BONE VARIABLES AT EACH TIME POINT
FOR '1-Tx'AND '2-Tx' GROUPS

Treatment Group	Bone Variable	6 Months Mean % Change (SD)	p-value	12 Months Mean % Change (SD)	p-value	18 Months Mean % Change (SD)	p-value	24 Months Mean % Change (SD)	p-value
1-Tx (n=72)	TOT-vBD	0.5	0.507	0.9	0.240	1.0	0.249	1.0	0.254
	TRAB-vBD	-2.2	0.003	-2.5	0.002	-3.6	0.000	-3.4	0.000
	CRT-vBD	1.1	0.235	1.8	0.193	2.8	0.010	2.4	0.037
	TOT-CSA	-1.7	0.006	-1.5	0.033	-2.5	0.004	-1.6	0.019
	TRAB-CSA	-2.2	0.008	-2.1	0.020	-2.3	0.011	-2.0	0.023
	CRT-CSA	-0.4	0.432	0.1	0.795	-1.3	0.028	-0.5	0.302
	TOT-CNT	-1.4	0.001	-0.9	0.013	-1.8	0.004	-0.9	0.018
	TRAB-CNT	-4.2	0.000	-4.3	0.001	-5.6	0.000	-5.2	0.000
	CRT-CNT	0.3	0.635	1.2	0.089	1.1	0.204	1.4	0.092
2-Tx (n=19)	TOT-vBD	3.2	0.010	2.5	0.009	3.5	0.004	2.5	0.107
	TRAB-vBD	-0.7	0.727	-1.7	0.204	-1.0	0.702	-1.2	0.449
	CRT-vBD	5.5	0.005	4.2	0.013	4.8	0.007	4.5	0.049
	TOT-CSA	-1.8	0.105	-1.8	0.042	-1.7	0.195	-1.3	0.207
	TRAB-CSA	-1.3	0.261	-2.2	0.027	-2.1	0.222	-1.2	0.258
	CRT-CSA	-2.3	0.093	-1.2	0.415	-0.9	0.363	-1.3	0.309
	TOT-CNT	1.1	0.122	0.5	0.394	1.5	0.126	1.0	0.616
	TRAB-CNT	-2.0	0.423	-3.8	0.043	-2.5	0.442	-2.2	0.258
	CRT-CNT	2.7	0.000	2.6	0.001	3.6	0.000	2.7	0.100

6.3.1.3   Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

Each of the six groups were evaluated independently for the 3 pQCT variables

(vBD, CNT and CSA) for each of the three compartments (TOT, CRT and TRAB) giving

9 pQCT variables measured in each treatment group (see TABLES 6.7-6.9). All of the 5

active therapy groups responded in a similar fashion for 8 of the 9 pQCT variables, with

TOT-CNT being the exception. For example, all 5 treatment groups generally increased

in 3 variables (TOT-vBD, CRT-vBD and CRT-CNT), while decreasing in the other 5

(TRAB-vBD, TRAB-CSA, TRAB-CNT, TOT-CSA and CRT-CSA) over the trial (see

FIGURES 6.11-6.13 in section 6.3.2.3). Although not all changes were statistically

significant in all groups for each variable at each timepoint, the direction of change versus

baseline was consistent across groups for the length of the trial. When considering TOT-

CNT, the two combination therapy groups (A+H and E+H) showed trends for gaining

bone, while the HRT, ALN and ETD single therapy groups appeared to lose total bone

content, similar to the control group.

TABLE 6.7

MEAN PERCENT CHANGES FROM BASELINE FOR PQCT RADIAL BONE DENSITY AT EACH TIME POINT FOR ALL SIX INDIVIDUAL TREATMENT GROUPS

Bone Variable	Treatment Group	6 Months Mean % Change (SD)	p-value	12 Months Mean % Change (SD)	p-value	18 Months Mean % Change (SD)	p-value	24 Months Mean % Change (SD)	p-value
TOT-vBD	A + H (n=9)	3.1 (4.8)	0.149	1.7 (4.3)	0.314	2.5 (5.5)	0.279	4.0 (6.4)	0.150
	ALN (n=24)	-0.9 (3.7)	0.178	1.3 (5.9)	0.341	0.6 (6.3)	1.000	1.1 (5.6)	0.518
	CNTL (n=32)	-0.4 (3.7)	0.530	-0.9 (4.5)	0.209	-0.8 (5.0)	0.294	-1.9 (5.7)	0.052
	E + H (n=10)	3.2 (4.0)	0.037	3.2 (3.2)	0.009	4.4 (3.1)	0.002	1.2 (6.0)	0.450
	ETD (n=27)	0.6 (5.4)	0.711	0.2 (6.3)	0.939	0.6 (5.2)	0.698	0.5 (6.5)	0.877
	HRT (n=21)	1.9 (4.7)	0.071	1.5 (4.7)	0.150	2.0 (4.9)	0.072	1.6 (4.6)	0.119
TRAB-vBD	A + H (n=9)	-2.3 (11.2)	0.498	-2.2 (9.5)	0.429	-2.0 (15.4)	0.689	0.1 (11.0)	0.937
	ALN (n=24)	-1.4 (7.4)	0.158	-0.8 (8.3)	0.193	-2.9 (6.4)	0.024	-2.5 (5.3)	0.025
	CNTL (n=32)	-0.5 (8.4)	0.522	-1.2 (6.4)	0.111	-0.6 (6.6)	0.263	-1.3 (7.8)	0.157
	E + H (n=10)	0.7 (4.4)	0.625	-1.2 (3.0)	0.206	-0.2 (4.0)	0.955	-2.3 (5.4)	0.233
	ETD (n=27)	-1.6 (7.5)	0.119	-2.0 (7.7)	0.140	-3.4 (6.8)	0.013	-3.9 (7.6)	0.021
	HRT (n=21)	-3.8 (6.9)	0.020	-5.1 (8.0)	0.009	-4.7 (8.7)	0.019	-3.6 (8.6)	0.036
CRT-vBD	A + H (n=9)	8.0 (8.6)	0.025	4.9 (8.3)	0.154	5.2 (9.1)	0.179	7.4 (10.5)	0.089
	ALN (n=24)	-0.8 (5.6)	0.511	2.4 (10.7)	0.388	2.0 (7.5)	0.380	2.7 (9.0)	0.222
	CNTL (n=32)	0.0 (6.0)	0.939	0.8 (6.6)	0.538	-0.1 (6.6)	0.871	-0.7 (7.9)	0.514
	E + H (n=10)	3.2 (5.5)	0.114	3.5 (4.8)	0.039	4.5 (3.6)	0.004	1.9 (6.1)	0.368
	ETD (n=27)	1.5 (10.0)	0.509	0.3 (11.2)	0.838	2.5 (10.0)	0.270	1.9 (9.4)	0.472
	HRT (n=21)	2.9 (6.8)	0.057	3.2 (8.8)	0.093	4.3 (7.3)	0.013	2.7 (7.0)	0.083

TABLE 6.8

MEAN PERCENT CHANGES FROM BASELINE FOR PQCT DISTAL RADIUS CROSS-SECTIONAL AREA AT EACH TIME POINT FOR ALL SIX INDIVIDUAL TREATMENT GROUPS

Bone Variable	Treatment Group	6 Months		12 Months		18 Months		24 Months	
		Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
TOT-CSA	A + H (n=9)	-2.0 (6.3)	0.264	-1.2 (3.9)	0.279	-0.7 (8.4)	0.719	-1.3 (8.1)	0.428
	ALN (n=24)	-0.6 (3.6)	0.409	-1.6 (7.1)	0.265	-2.2 (6.2)	0.082	-2.0 (6.0)	0.118
	CNTL (n=32)	-0.8 (3.9)	0.329	-1.2 (4.0)	0.146	-0.4 (4.7)	0.610	0.1 (5.2)	0.769
	E + H (n=10)	-1.7 (5.4)	0.276	-2.4 (3.7)	0.096	-2.7 (4.9)	0.067	-1.3 (3.2)	0.246
	ETD (n=27)	-2.1 (5.9)	0.058	-1.0 (5.8)	0.257	-1.5 (5.3)	0.128	-1.4 (6.0)	0.193
	HRT (n=21)	-2.4 (5.4)	0.071	-1.9 (5.0)	0.122	-4.2 (9.6)	0.090	-1.4 (4.7)	0.274
TRAB-CSA	A + H (n=9)	-0.2 (7.6)	0.697	-0.6 (3.2)	0.354	0.0 (10.7)	0.892	-0.5 (8.9)	0.576
	ALN (n=24)	-0.6 (4.6)	0.459	-1.8 (9.1)	0.288	-2.5 (8.3)	0.108	-1.9 (7.0)	0.176
	CNTL (n=32)	-0.4 (7.0)	0.664	0.1 (7.1)	0.940	0.6 (7.9)	0.852	1.6 (8.5)	0.377
	E + H (n=10)	-2.3 (7.3)	0.255	-3.6 (4.8)	0.049	-3.9 (8.5)	0.076	-1.8 (5.0)	0.278
	ETD (n=27)	-2.8 (7.2)	0.044	-2.1 (8.1)	0.127	-1.8 (6.2)	0.156	-2.1 (8.1)	0.138
	HRT (n=21)	-3.4 (8.6)	0.126	-2.6 (6.8)	0.131	-2.8 (7.3)	0.208	-2.0 (7.1)	0.320
CRT-CSA	A + H (n=9)	-4.4 (5.8)	0.048	-2.3 (7.5)	0.349	-1.7 (6.2)	0.380	-2.6 (8.1)	0.297
	ALN (n=24)	0.0 (6.8)	0.772	-0.5 (9.0)	0.579	-1.1 (5.6)	0.271	-1.5 (7.7)	0.228
	CNTL (n=32)	-0.7 (6.7)	0.427	-2.5 (7.5)	0.068	-1.3 (6.7)	0.213	-1.3 (8.0)	0.326
	E + H (n=10)	-0.3 (5.8)	0.795	-0.2 (3.7)	0.902	-0.1 (3.8)	0.812	-0.1 (5.0)	0.841
	ETD (n=27)	-0.7 (8.5)	0.578	1.0 (7.6)	0.548	-1.0 (6.9)	0.361	-0.1 (6.3)	0.837
	HRT (n=21)	-0.5 (7.0)	0.639	-0.4 (8.0)	0.687	-2.1 (5.2)	0.056	0.0 (6.6)	0.843

TABLE 6.9

MEAN PERCENT CHANGES FROM BASELINE FOR PQCT RADIAL BONE CONTENT AT EACH TIME POINT FOR ALL SIX INDIVIDUAL TREATMENT GROUPS

Bone Variable	Treatment Group	6 Months		12 Months		18 Months		24 Months	
		Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value	Mean % Change (SD)	p-value
TOT-CNT	A + H (n=9)	0.9 (4.6)	0.392	0.4 (4.1)	0.719	1.5 (6.2)	0.412	2.3 (5.6)	0.228
	ALN (n=24)	-1.5 (3.0)	0.026	-0.7 (3.9)	0.279	-1.9 (3.9)	0.029	-1.2 (3.0)	0.041
	CNTL (n=32)	-1.3 (2.3)	0.002	-2.2 (3.2)	0.000	-1.4 (3.6)	0.019	-2.0 (4.0)	0.005
	E + H (n=10)	1.3 (2.5)	0.166	0.7 (2.0)	0.320	1.5 (2.7)	0.098	-0.2 (6.0)	0.804
	ETD (n=27)	-1.8 (4.1)	0.051	-1.2 (2.6)	0.042	-1.1 (2.9)	0.052	-1.3 (3.5)	0.080
	HRT (n=21)	-0.8 (2.8)	0.220	-0.7 (2.8)	0.239	-2.5 (8.6)	0.173	0.0 (3.3)	0.853
TRAB-CNT	A + H (n=9)	-2.3 (15.4)	0.652	-2.7 (10.4)	0.356	-0.9 (23.1)	0.909	0.0 (16.4)	0.860
	ALN (n=24)	-1.9 (9.3)	0.107	-2.3 (15.2)	0.112	-5.2 (10.9)	0.010	-4.5 (8.1)	0.006
	CNTL (n=32)	-0.7 (13.6)	0.429	-1.0 (10.4)	0.240	0.2 (12.0)	0.690	0.6 (15.3)	0.797
	E + H (n=10)	-1.7 (6.8)	0.495	-4.7 (6.2)	0.054	-4.0 (10.1)	0.155	-4.2 (6.9)	0.099
	ETD (n=27)	-4.2 (11.4)	0.021	-3.7 (13.4)	0.095	-4.9 (11.1)	0.021	-5.7 (12.8)	0.023
	HRT (n=21)	-6.8 (12.2)	0.022	-7.4 (11.6)	0.007	-7.1 (12.9)	0.025	-5.4 (12.5)	0.061
CRT-CNT	A + H (n=9)	2.8 (3.4)	0.040	2.0 (2.5)	0.040	2.9 (4.6)	0.098	3.9 (3.3)	0.013
	ALN (n=24)	-1.1 (4.3)	0.242	1.1 (5.0)	0.392	0.7 (6.0)	0.852	0.6 (4.1)	0.563
	CNTL (n=32)	-0.9 (4.9)	0.237	-2.1 (5.1)	0.015	-1.6 (5.9)	0.079	-2.3 (6.5)	0.029
	E + H (n=10)	2.6 (2.3)	0.004	3.2 (2.8)	0.008	4.3 (2.4)	0.000	1.7 (7.2)	0.591
	ETD (n=27)	0.2 (6.8)	0.885	0.7 (6.2)	0.704	1.0 (5.8)	0.484	1.4 (7.0)	0.453
	HRT (n=21)	2.0 (5.3)	0.100	2.2 (4.8)	0.060	1.9 (5.7)	0.199	2.4 (5.9)	0.124

6.3.2 PQCT: Comparison of Different Treatment Regimens

Repeated measures ANOVAs were used to examine if any differences existed between treatments for each of the pQCT bone variables over the 2-year study. Post-hoc tests for repeated measures ANOVA were used to determine between which treatments the differences existed. In order to check at which time point differences between treatments existed, unpaired t-tests and one-way ANOVA was used for both the percent change from baseline and the area under the curve (AUC) from baseline. Post-hoc tests for one-way ANOVA were used to identify which treatments were different at each time point. For further details see section 4.4.3.3.

6.3.2.1 Cohort Divided into 2 Groups: 'Any Treatment' and Control

Repeated measures ANOVA detected differences between the 'Any-Tx' and control groups for the four pQCT variables TOT-vBD, TRAB-CSA, TRAB-CNT and CRT-CNT over the two-year trial (see TABLE 6.10 and FIGURES 6.5-6.7). One-way ANOVA tests using percent change and AUC values showed similar results. Both methods determined that the 'Any-Tx' group gained significantly more TOT-vBD than the control group by 12, 18 and 24 months (2.2-3.2% treatment effect, p-values 0.009 to 0.029). The same pattern was found for CRT-CNT with treatment effects of 3.3-4.0% (p-values 0.002 to 0.010). The 'Any-Tx' group lost significantly more trabecular area than the control group at the 24-month mark (3.5% difference between groups, p<0.05 and p=0.057 for percent change and AUC analyses, respectively). Trabecular content showed similar changes with

the 'Any-Tx' group losing 5.2% more trabecular bone than the control group by 18

($p<0.05$ using percent change values) and 24 months ($p<0.05$ using AUC values).

TABLE 6.10

PQCT TREATMENT EFFECT AND T-TEST RESULTS (P-VALUE) FOR RADIAL BONE VARIABLES
AT EACH TIME POINT FOR THE 'ANY TREATMENT' GROUP VERSUS THE 'CONTROL' GROUP

pQCT Variable	6 Months		12 Months		18 Months		24 Months	
	Tx Effect*	p-value	Tx Effect	p-value	Tx Effect	p-value	Tx Effect	p-value
TOT-vBD	1.43	0.087	2.19	0.029	2.35	0.029	3.19	0.009
TRAB-vBD	-1.39	0.413	-1.12	0.428	-2.44	0.094	-1.57	0.326
CRT-vBD	2.03	0.137	1.47	0.347	3.30	0.025	3.51	0.038
TOT-CSA	-0.90	0.307	-0.39	0.670	-2.00	0.073	-1.67	0.129
TRAB-CSA	-1.61	0.271	-2.22	0.140	-2.92	0.075	-3.46	0.045
CRT-CSA	-0.12	0.933	2.37	0.133	0.08	0.950	0.58	0.717
TOT-CNT	0.44	0.426	1.63	0.016	0.25	0.768	1.47	0.081
TRAB-CNT	-3.09	0.253	-3.16	0.169	-5.20	0.042	-5.16	0.088
CRT-CNT	1.75	0.096	3.61	0.001	3.25	0.008	4.04	0.003

*Tx Effect (Treatment Effect) = % change for 'Any-Tx' group - % change for Control group

FIGURE 6.5

PERCENT CHANGES FOR CONTROL AND 'ANY-TX' GROUPS OVER 2-YEARS
IN A) TOT-vBD, B) TOT-CSA and C) TOT-CNT
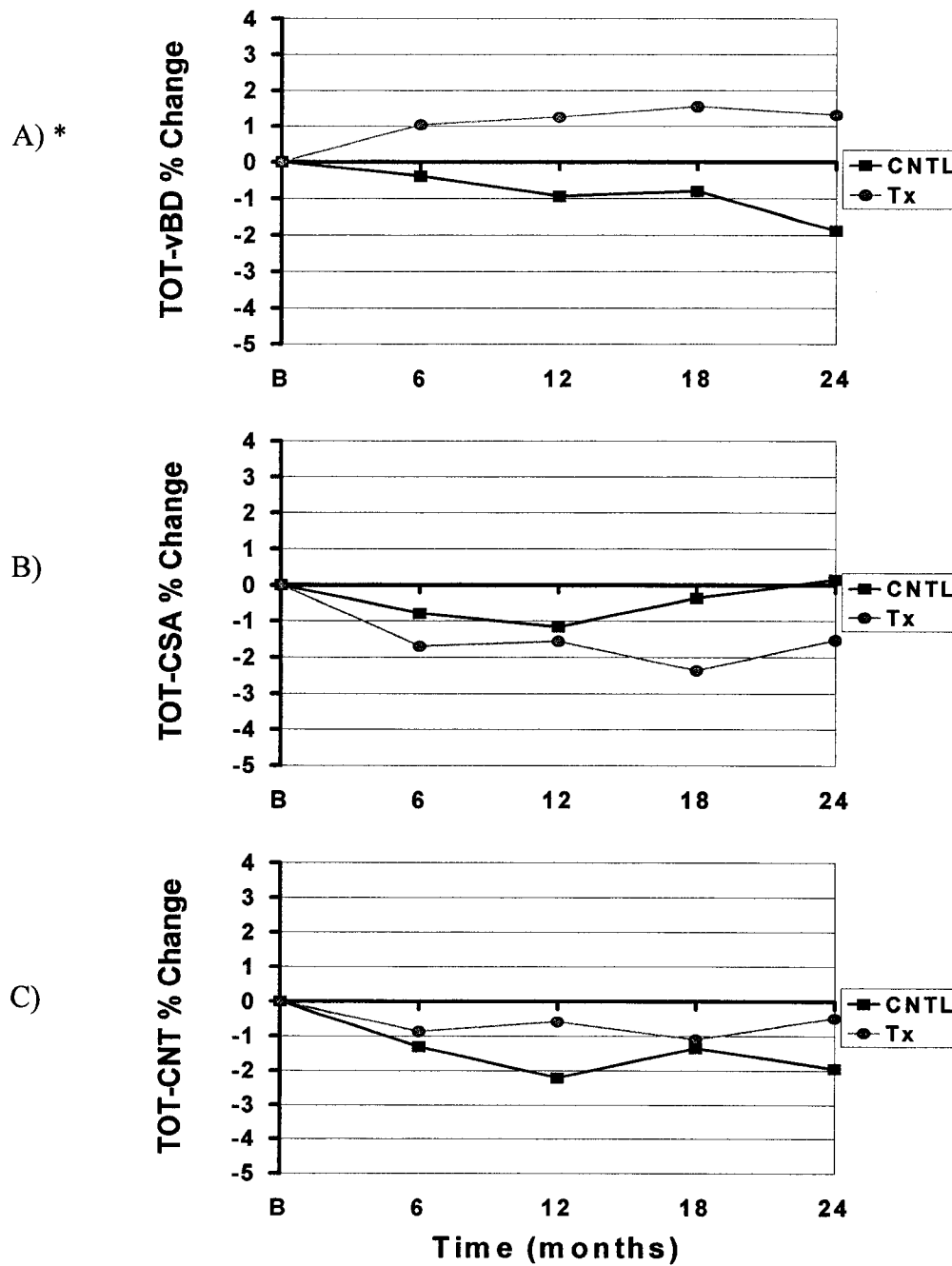
* Significant difference between 'Any-Tx' and Control with repeated measures ANOVA

FIGURE 6.6

PERCENT CHANGES FOR CONTROL AND 'ANY-TX' GROUPS OVER 2-YEARS
IN A) TRAB-vBD, B) TRAB-CSA and C) TRAB-CNT

* Significant difference between 'Any-Tx' and Control with repeated measures ANOVA

FIGURE 6.7

PERCENT CHANGES FOR CONTROL AND 'ANY-TX' GROUPS OVER 2-YEARS
IN A) CRT-vBD, B) CRT-CSA and C) CRT-CNT

A)

B)

C) *

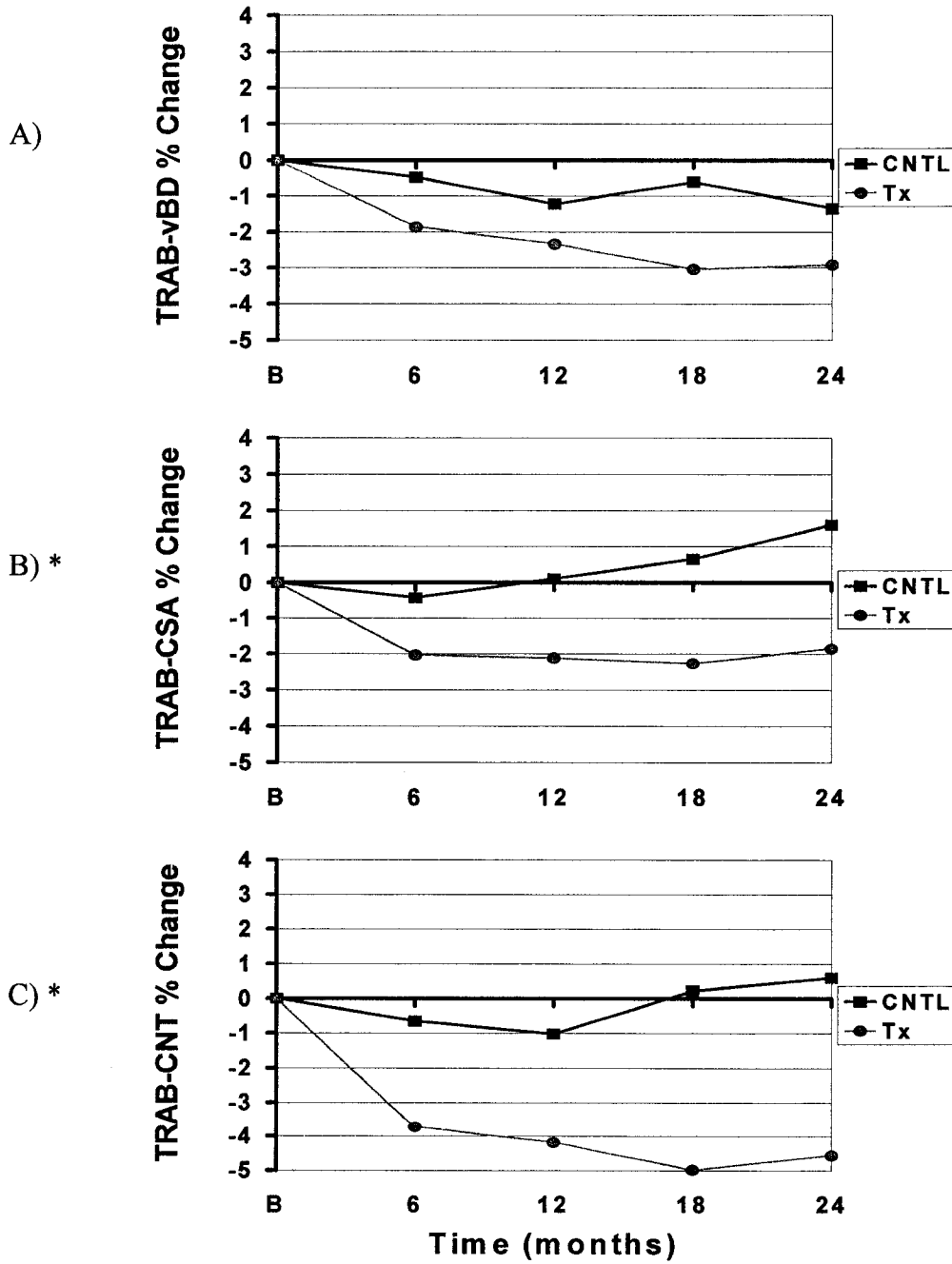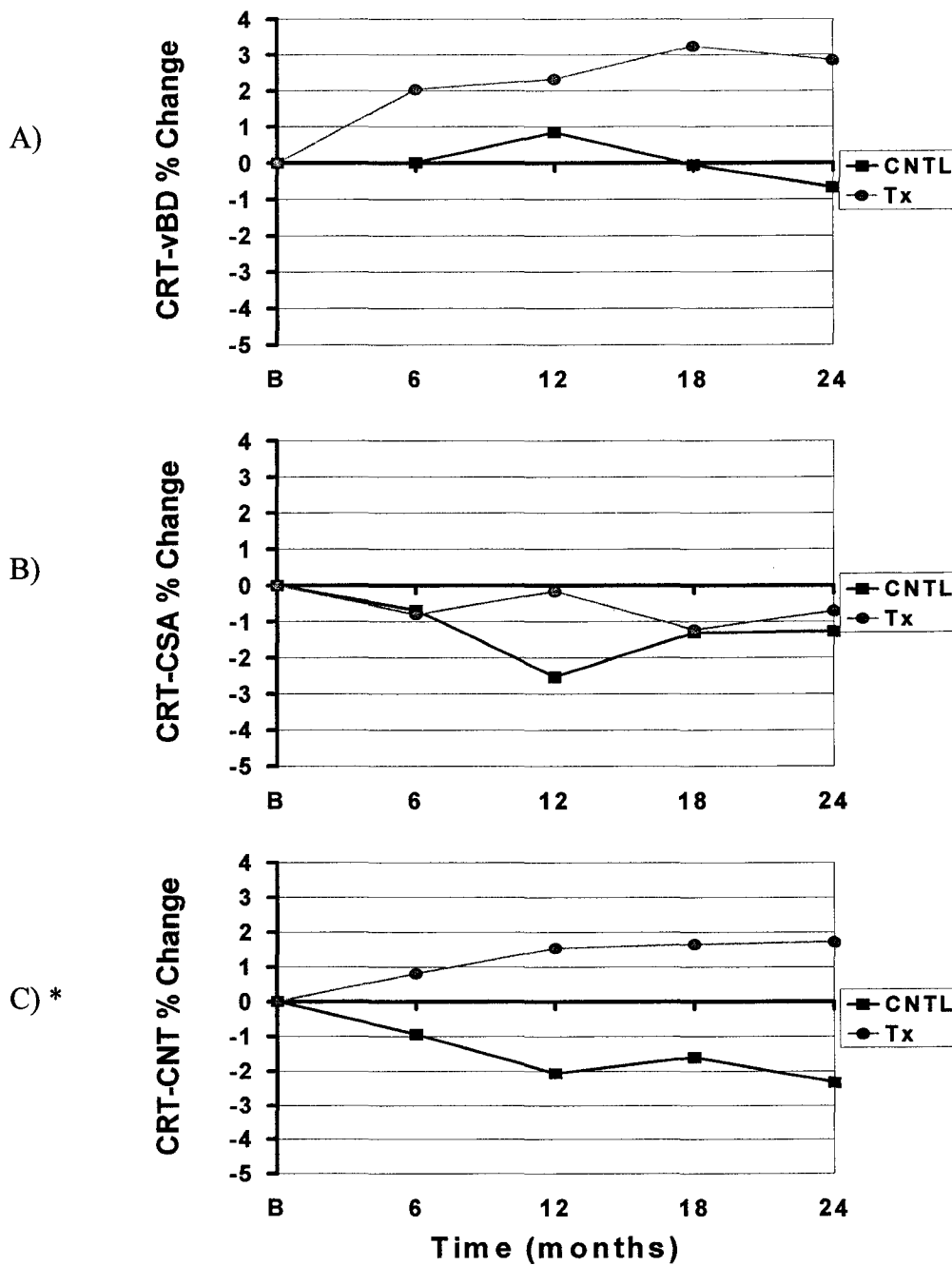* Significant difference between 'Any-Tx' and Control with repeated measures ANOVA

6.3.2.2 Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

Repeated measures ANOVA detected differences between groups for total and

cortical volumetric bone density as well as total and cortical bone content over the two-

year trial. Post-hoc testing determined that the '2-Tx' group had greater increases in TOT-

vBD and CRT-vBD than the control group over the two years. For total content, the '2-

Tx' group had gained significantly more bone than both the control group and the '1-Tx'

group. In the cortical compartment, the '2-Tx' and '1-Tx' groups both gained

significantly more bone content than the control group, but there was no significant

difference between the amount of cortical bone content gained by the two active therapy

regimens. Trabecular content showed a trend towards the '1-Tx' group losing more bone

than the control group (p=0.053) over the two years (see FIGURES 6.8-6.10).

One-way ANOVA analysis by AUC and percent change showed very similar

results. These analyses found significant differences between groups for changes in TOT-

vBD at all time points (6, 12, 18 and 24 months). Post-hoc testing determined that the '2-

Tx' group had gained significantly more total density than the control group at all time

points (treatment effects from 3.4 to 4.4%). Significant differences between groups for

cortical density were only detected at the 6-month mark with post-hoc testing determining

that the '2-Tx' group gained 5.5% more density in the cortex than the control group. All

other time points had trends towards differences in cortical density changes between the

'2-Tx' and control groups and post-hoc testing (Dunnett's test) found these differences to

be significant at 12 and 24 months. The '2-Tx' group gained significantly more total bone

content than both the control (2.4-3.0% more than control) and '1-Tx' (1.9-3.3% more

than '1-Tx') groups at all time points except 6 months, where significant differences were

only found between the '2-Tx' and control groups. The '2-Tx' group also gained

significantly more cortical bone content than the control group (treatment effects from 3.7

to 5.2%) at all time points. At the 18-month and 24-month time points, the '1-Tx' group

had also gained significantly more cortical bone content (2.7% and 3.8% respectively)

than the control group.

FIGURE 6.8

PERCENT CHANGES FOR CONTROL, '1-TX' AND '2-TX' GROUPS OVER 2-YEARS in A) TOT-vBD and B) TOT-CNT
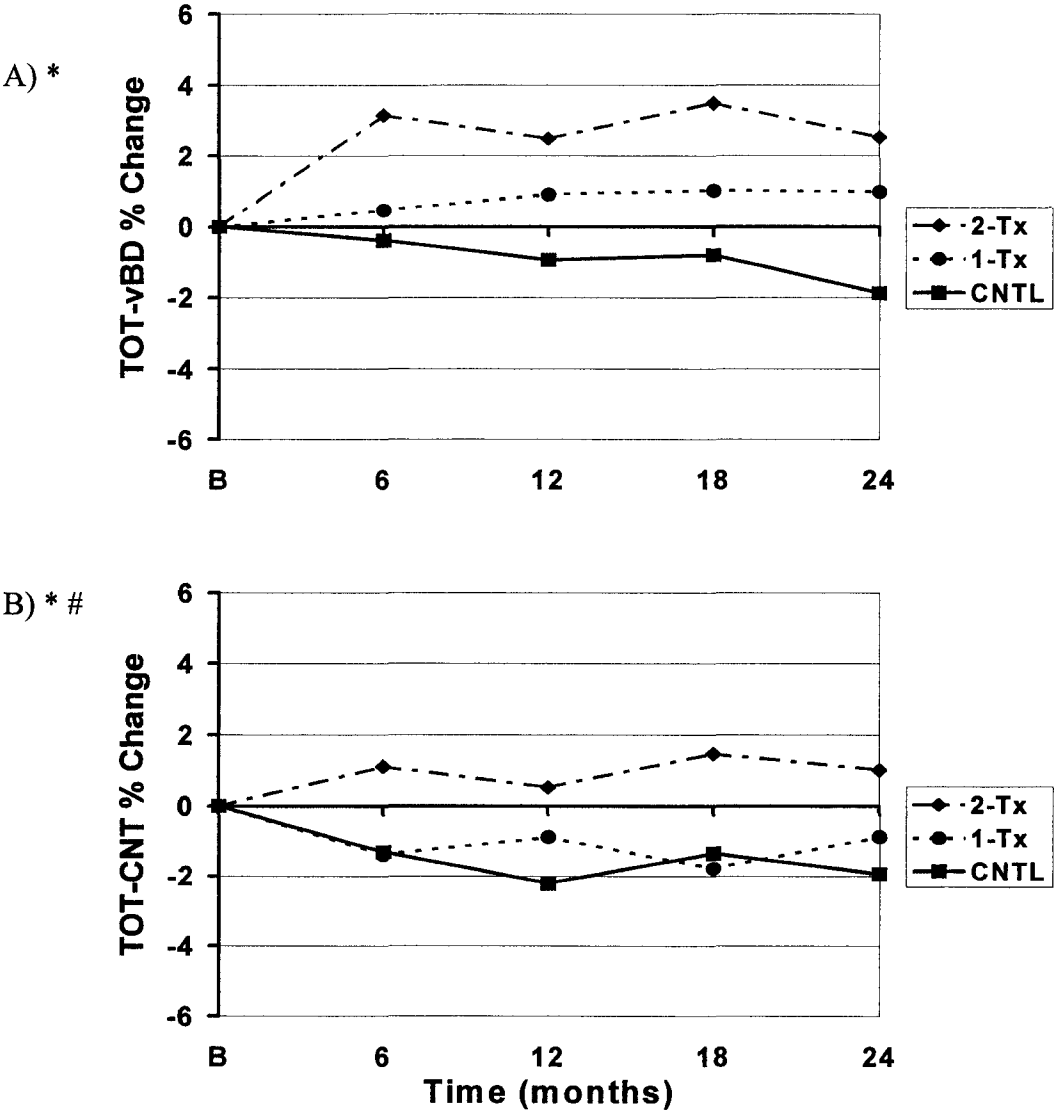
* Significant difference between '2-Tx' and Control with repeated measures ANOVA
# Significant difference between '2-Tx' and '1-Tx' with repeated measures ANOVA

FIGURE 6.9

PERCENT CHANGES FOR CONTROL, '1-TX' AND '2-TX' GROUPS OVER 2-YEARS in A) TRAB-vBD and B) TRAB-CNT

$ Borderline significant difference between '1-Tx' and Control with repeated measures ANOVA, p=0.053
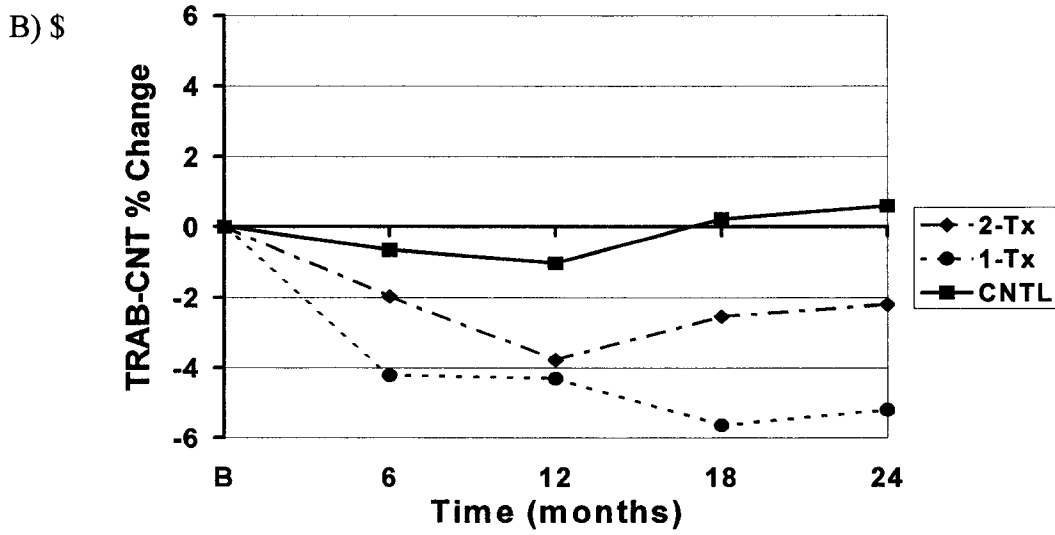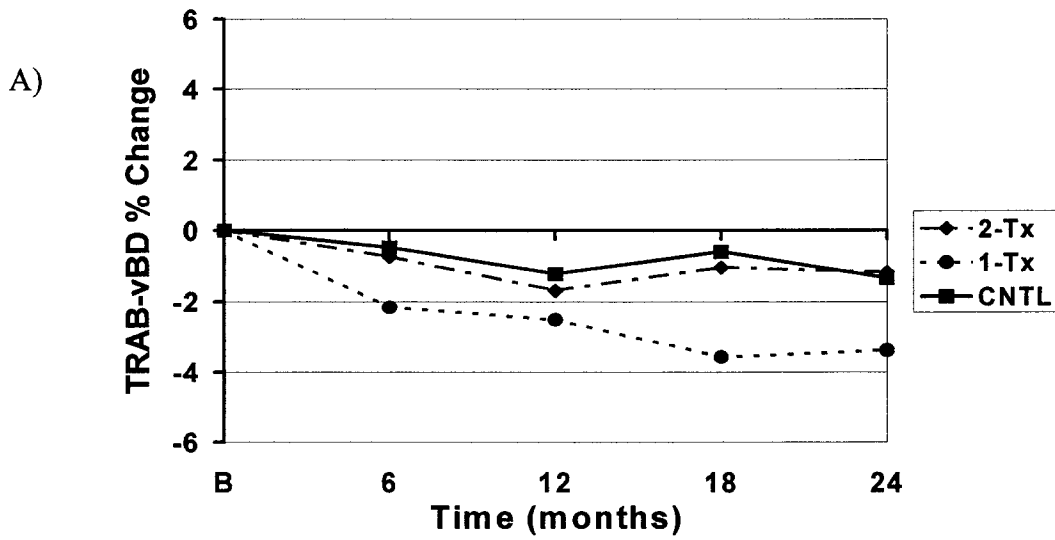
FIGURE 6.10

PERCENT CHANGES FOR CONTROL, '1-TX' AND '2-TX' GROUPS OVER 2-YEARS in A) CRT-vBD and B) CRT-CNT
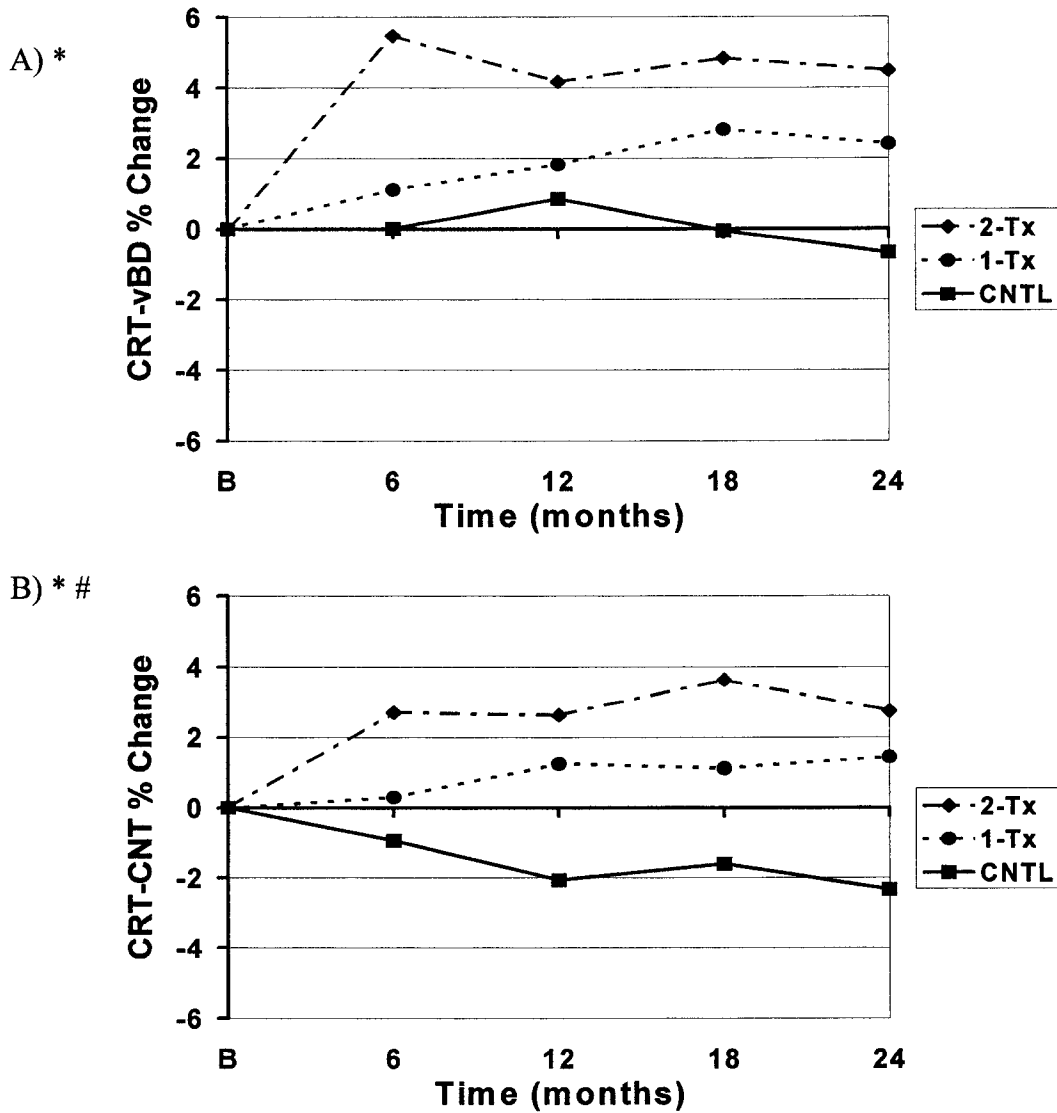
A) *

B) * #

* Significant difference between '2-Tx' and Control with repeated measures ANOVA
# Significant difference between '2-Tx' and '1-Tx' with repeated measures ANOVA

6.3.2.3   Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

Repeated measures ANOVA for each of the nine pQCT variables were performed with the subjects divided into the 6 individual treatment groups. These tests found differences between groups for total and cortical content (p<0.05) and was borderline significant for total bone density (p=0.057). Post-hoc testing on the repeated measures showed that the A+H treatment gained significantly more total content at the distal radius than the control group over the two years and the E+H group showed a trend towards gaining more total bone content (p=0.09) than the control group. Post-hoc testing on the repeated measures for cortical bone content showed that the A+H, E+H and HRT groups all gained significantly more bone at the cortex than the control group. The trend for differences in total bone density changes from baseline was between the E+H and control groups, with the E+H group gaining more total bone density than the control group (p=0.057).

One-way ANOVA tests supported the repeated measures ANOVA findings with significant differences between groups detected for TOT-vBD, TOT-CNT and CRT-CNT at different time points. Significant differences between groups for total bone density AUC and % changes from baseline were seen at 6 months and trends for differences between groups were found at 12 through to 24 months (p-values 0.053-0.057) with AUC analysis. Post-hoc testing identified that, in general, the E+H group gained more total radial bone density than the control group. Total radial bone content behaved comparably with significant differences detected at 18 and 24 months and trends

for differences between groups at 6 and 12 months (P=0.063 and 0.055 respectively) with AUC values. When percent change in total content was utilized, only trends for differences between groups were seen (p=0.059, 0.091 and 0.081 for 6, 12 and 24 months respectively). One-way ANOVA of cortical radial bone content measurements showed significant differences existed between groups for AUC and percent changes at 12, 18 and 24-month time points (p<0.05). A trend towards differences between groups was also seen at 6 months with both methods (p=0.080). Post-hoc analysis revealed that the differences in cortical content changes from baseline involved the HRT, E+H and A+H groups gaining significantly more bone than the control group at different time points. E+H and HRT groups were consistently found to have gained more cortical bone than the control group from the 12-month mark onward, while A+H gains were only detected to be significantly different from the control group at 24 months. In addition to these differences in cortical content, one-way ANOVA on both the AUC and % changes from baseline at 6 months detected differences between the A+H and ALN group, which were not found with repeated measures ANOVA.

See FIGURES 6.11-6.13 for examples of changes in pQCT variables (TOT-vBD, TRAB-CSA and CRT-CNT) by control group and each of the 5 separate treatment groups.

FIGURE 6.11

PERCENT CHANGES FOR CONTROL AND 5 DIFFERENT TREATMENT GROUPS OVER 2-YEARS IN TOT-vBD
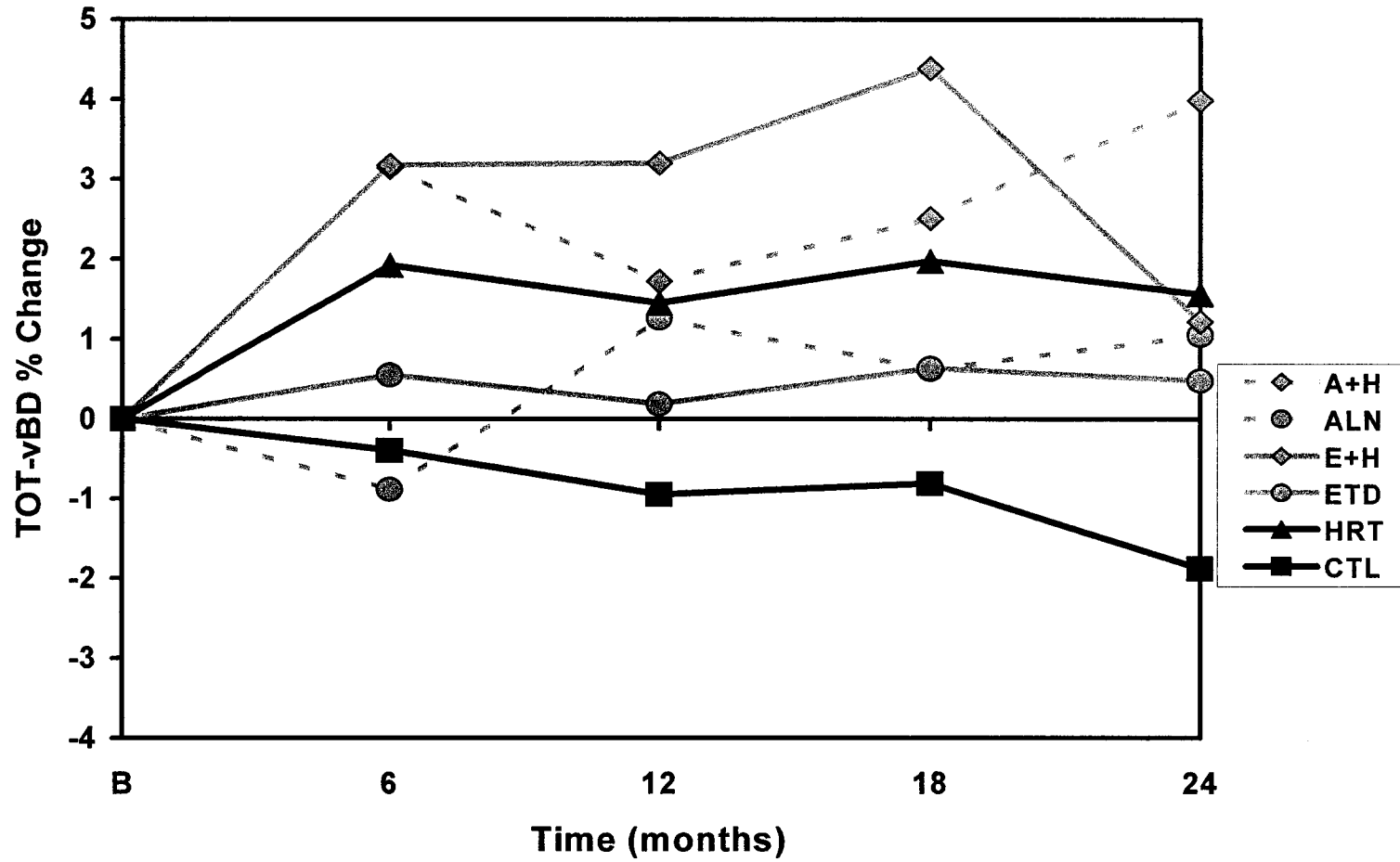
FIGURE 6.12

PERCENT CHANGES FOR CONTROL AND 5 DIFFERENT TREATMENT GROUPS OVER 2-YEARS IN TRAB-CSA
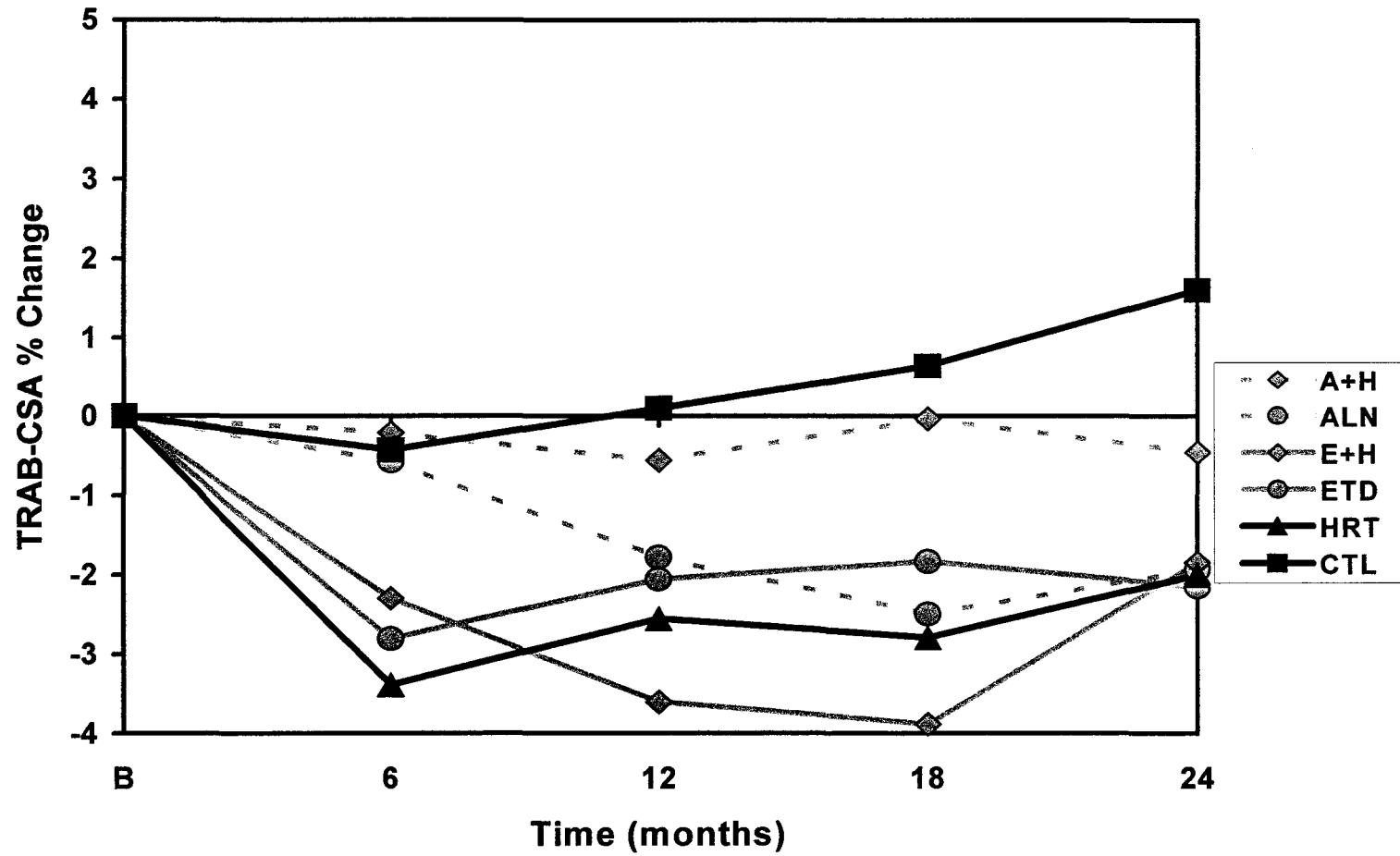
FIGURE 6.13

PERCENT CHANGES FOR CONTROL AND 5 DIFFERENT TREATMENT GROUPS OVER 2-YEARS IN CRT-CNT
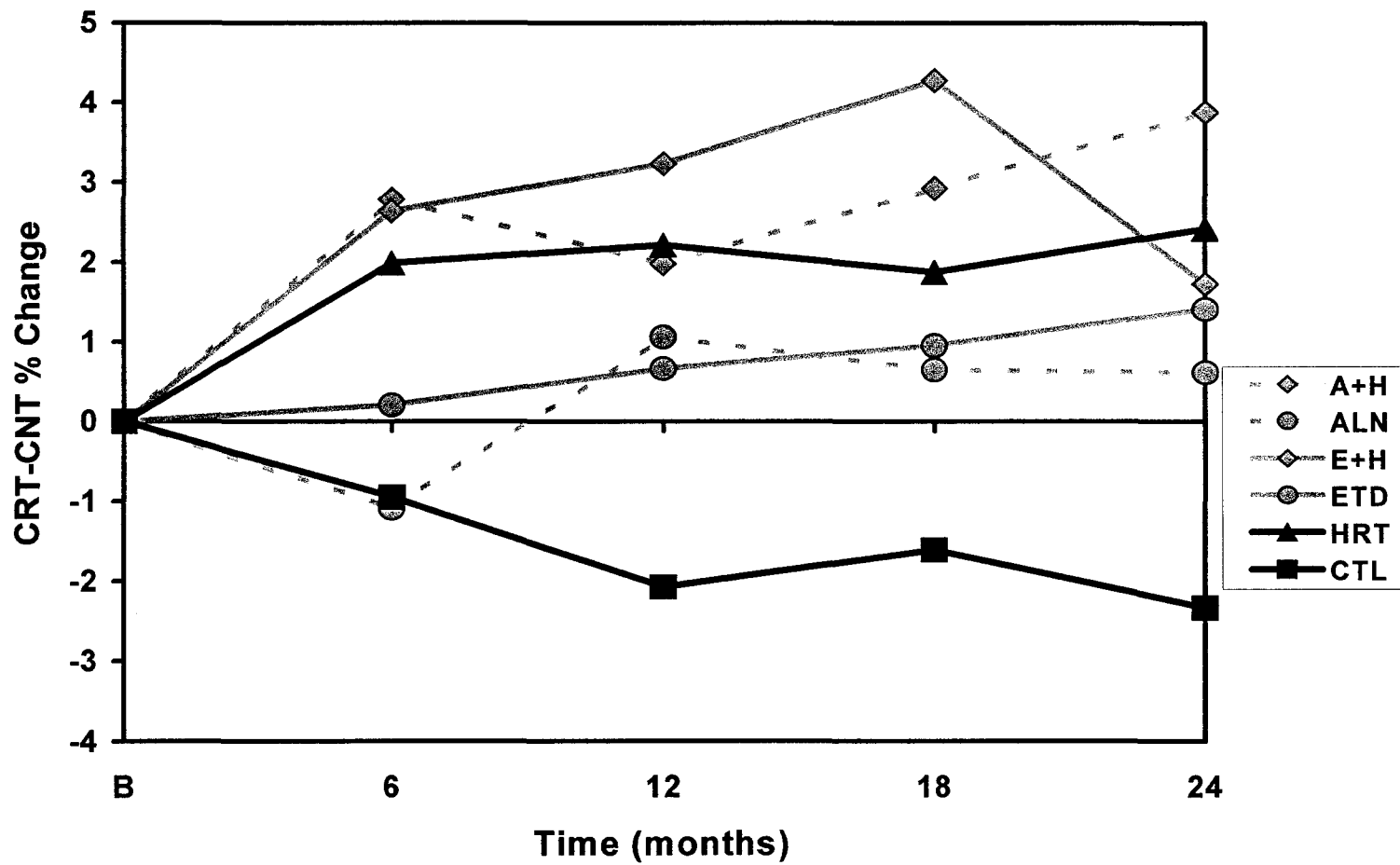
6.4   Trabecular Structure

6.4.1   Structure: Changes from Baseline

The total cohort was divided into treatment regimens by three different sets of

criteria as described for DXA (section 6.2.1). Percent changes from baseline to each of 6,

12, 18, 24 months were computed for each subject, and the mean absolute changes were

calculated for each of the different treatment regimens (see Tables 6.11-6.12). Paired t-

tests for each trabecular structure variable compared absolute values at each time point (6,

12, 18 and 24-months) to corresponding baseline values to determine if significant

changes had occurred.

6.4.1.1   Cohort Divided into 2 Groups: 'Any Treatment' and Control

Changes in trabecular structure indices were subtle, with few statistically significant

differences from baseline. Trends in the data suggest that the control group may have

increased in maximum hole size (Hm) and that the treatment group may have lost some

trabecular connectivity (CI) over the two-year trial. The 'Tx' group showed a consistent

trend for a loss in connectivity, but this loss only reached significance at 18 months with a

1.11 decrease in C.I. (p=0.009) versus the group's mean baseline value. The connectivity

index in the control group decreased slightly at 6 months, but values were near baseline

levels for the rest of the trial. The maximum hole size remained constant in the treatment

group throughout the trial. The control group showed early signs of an increase in Hm

with gains at 6 and 12 months approaching significance (p=0.071 and 0.057,

respectively), but values at 18 and 24 months were back down near baseline values.

Average hole size (Ha) remained relatively unchanged from baseline for every time point

in the trial for both the treatment and control groups (see TABLE 6.11).

6.4.1.2 Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

The division of the treatment group into '1-Tx' and 2-'Tx' groups showed that the '1-

Tx' group was more consistent than the '2-Tx'. The Ha remained relatively stable for both

groups, with the '2-Tx' group suggesting a small increase in size at every time point in the

trial, but this did not reach significance at any time (greatest difference from baseline was

1.33 mm^2, p=0.224 at 18 months). Similarly, there were no significant differences from

baseline for either the '1-Tx' or '2-Tx' groups for Hm. The '1-Tx' group appeared more

stable, with Hm values remaining very close to baseline values for the entire trial. When

C.I. was considered, the '1-Tx' group had a lower C.I. at every time point versus baseline

values and was significantly lower by 1.10 at 18 months (p=0.011). The '2-Tx' group had

slightly greater losses in C.I. at 18 and 24 months, but no value at any time point was

found to be significantly different from baseline for the '2-Tx' group (see TABLE 6.11).

TABLE 6.11

MEAN ABSOLUTE CHANGES FROM BASELINE FOR PQCT STRUCTURE AT EACH TIME POINT FOR
CONTROL, ANY-TREATMENT, 1-TX AND 2-TX GROUPS

Treatment Group	Structure Index	6 Months		12 Months		18 Months		24 Months	
		Mean Change (SD)	p-value	Mean Change (SD)	p-value	Mean Change (SD)	p-value	Mean Change (SD)	p-value
CNTL	Ha	0.24 (1.95)	0.494	-0.23 (1.53)	0.394	-0.26 (2.06)	0.488	-0.11 (3.10)	0.837
	Hm	4.34 (13.15)	0.071	4.26 (12.20)	0.057	0.54 (10.01)	0.760	-0.87 (14.66)	0.738
	CI	-0.96 (3.67)	0.151	-0.43 (3.74)	0.524	0.05 (4.35)	0.947	0.35 (5.64)	0.726
ANY-TX	Ha	0.27 (4.70)	0.586	0.56 (8.70)	0.541	0.18 (3.90)	0.658	0.30 (5.27)	0.586
	Hm	-0.33 (13.30)	0.815	-0.04 (13.27)	0.976	-0.76 (13.36)	0.591	0.64 (16.04)	0.706
	CI	-0.52 (4.23)	0.247	-0.52 (4.08)	0.231	-1.11 (4.00)	0.009	-0.73 (4.19)	0.100
1-TX	Ha	0.24 (5.04)	0.685	0.60 (9.69)	0.600	-0.12 (3.68)	0.782	0.10 (5.47)	0.882
	Hm	-0.10 (12.94)	0.946	-0.28 (12.40)	0.850	-0.20 (12.70)	0.893	-0.30 (12.10)	0.837
	CI	-0.71 (4.22)	0.160	-0.43 (4.02)	0.372	-1.10 (3.57)	0.011	-0.53 (4.03)	0.265
2-TX	Ha	0.37 (3.22)	0.620	0.40 (2.77)	0.543	1.33 (4.59)	0.224	1.08 (4.48)	0.306
	Hm	-1.18 (14.92)	0.734	0.85 (16.54)	0.825	-2.85 (15.81)	0.442	4.17 (26.31)	0.499
	CI	0.20 (4.33)	0.846	-0.86 (4.39)	0.405	-1.18 (5.45)	0.359	-1.47 (4.80)	0.198

6.4.1.3   Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

Once the subjects on therapy were grouped according to the 5 different active treatments, there were still very few significant changes relative to baseline values for any of the trabecular structure measurements. No significant differences were found for any of the five individual treatment groups for either Ha or Hm. ALN suggested a 1.92 mm^2 decrease in Ha at 24 months (p=0.061). HRT approached a significant gain in Ha at 18 months with a change of 0.59 mm^2 (p=0.077). The HRT group changes from baseline for Ha were larger at the 6, 12 and 24-month marks, but the p-values were also larger. Individual treatments did not produce significant changes from baseline values in C.I. except for the HRT group. HRT produced decreases in C.I. at all 4 time points and these reached significance at 6, 12 and 18 months (p<0.05 for all) and was nearing significance at 24 months (p=0.067). Losses in C.I. for HRT ranged from 1.95 to 2.46 over the 2-year trial (see TABLE 6.12).

TABLE 6.12

MEAN ABSOLUTE CHANGES FROM BASELINE FOR PQCT STRUCTURE AT EACH TIME POINT FOR
ALL FIVE INDIVIDUAL TREATMENT GROUPS

Treatment Group	Structure Index	6 Months		12 Months		18 Months		24 Months	
		Mean Change (SD)	p-value	Mean Change (SD)	p-value	Mean Change (SD)	p-value	Mean Change (SD)	p-value
Ha	A + H (n=9)	1.11 (4.56)	0.488	1.15 (3.91)	0.403	3.40 (5.95)	0.125	2.58 (6.04)	0.236
	ALN (n=24)	-0.70 (5.55)	0.542	-1.84 (6.14)	0.155	-1.22 (5.29)	0.268	-1.92 (4.78)	0.061
	CNTL (n=32)	0.24 (1.95)	0.494	-0.23 (1.52)	0.394	-0.26 (2.06)	0.488	-0.11 (3.10)	0.837
	E + H (n=10)	-0.29 (1.10)	0.431	-0.29 (0.84)	0.307	-0.54 (1.58)	0.304	-0.26 (1.86)	0.663
	ETD (n=27)	-0.22 (3.74)	0.765	0.06 (3.65)	0.936	0.31 (2.97)	0.592	0.23 (4.02)	0.769
	HRT (n=21)	1.91 (5.67)	0.138	4.10 (15.88)	0.251	0.59 (1.44)	0.077	2.23 (7.02)	0.161
Hm	A + H (n=9)	-0.37 (13.01)	0.934	0.58 (10.65)	0.874	-1.12 (12.16)	0.789	-2.61 (13.76)	0.585
	ALN (n=24)	-1.55 (11.59)	0.518	-3.36 (12.66)	0.207	-0.54 (14.12)	0.854	-2.65 (9.47)	0.184
	CNTL (n=32)	4.34 (13.15)	0.071	4.26 (12.20)	0.057	0.54 (10.01)	0.760	-0.87 (14.66)	0.738
	E + H (n=10)	-1.90 (17.14)	0.733	1.09 (21.12)	0.874	-4.41 (19.04)	0.483	10.30 (33.60)	0.359
	ETD (n=27)	-0.54 (13.90)	0.840	-0.08 (13.06)	0.976	-1.72 (14.24)	0.536	0.71 (14.50)	0.801
	HRT (n=21)	2.12 (13.46)	0.478	2.99 (10.80)	0.220	2.13 (8.40)	0.260	1.10 (11.53)	0.665
CI	A + H (n=9)	0.81 (5.54)	0.673	-1.71 (5.43)	0.372	-1.33 (7.27)	0.599	-0.95 (6.10)	0.655
	ALN (n=24)	-0.13 (3.73)	0.864	0.64 (3.50)	0.377	-0.84 (2.85)	0.165	0.52 (3.04)	0.415
	CNTL (n=32)	-0.96 (3.67)	0.151	-0.43 (3.74)	0.524	0.05 (4.35)	0.947	0.35 (5.64)	0.726
	E + H (n=10)	-0.36 (3.07)	0.721	-0.09 (3.31)	0.933	-1.04 (3.53)	0.375	-1.95 (3.53)	0.116
	ETD (n=27)	0.15 (3.72)	0.835	-0.09 (3.95)	0.907	-0.66 (3.80)	0.375	-0.18 (3.49)	0.793
	HRT (n=21)	-2.46 (4.96)	0.034	-2.08 (4.32)	0.039	-1.95 (3.99)	0.036	-2.19 (5.17)	0.067

6.4.2   Structure: Comparisons of Different Treatment Regimens

Repeated measures ANOVAs were used to examine if any differences existed between treatments over the 2-year trial for each of the pQCT trabecular structure variables. Post-hoc tests for repeated measures ANOVA were used to determine between which treatments the differences existed. In order to check at which time point differences between treatments existed, unpaired t-tests and one-way ANOVA was used for both the absolute change from baseline and the area under the curve (AUC) from baseline. Post-hoc tests for one-way ANOVA were used to identify which treatments were different at each time point. See section 4.4.3.3 for further details.

6.4.2.1   Cohort Divided into 2 Groups: 'Any Treatment' and Control

Repeated measures ANOVA found no significant difference between the treatment group and control group over the two-year trial for any of the three structure variables. Unpaired t-tests revealed that there were no significant differences between the 'Any-Tx' and control group for any structure variable at any time point. The differences between these two groups that were the closest to being statistically significant were Hm comparisons at 6 and 12 months. At 6 months, the control group had increased 4.67 mm^2 more than the treatment group in maximum hole size and 4.30 mm^2 at 12 months (p=0.091 and 0.099, respectively). AUC values showed very similar results with no differences between treatment and control groups for Ha or C.I. and trends in Hm at 6 months (p=0.089) and 12 months (p=0.073).

6.4.2.2   Cohort Divided into 3 Groups: '1-Tx', '2-Tx' and Control

Both the repeated measures ANOVA and the one-way ANOVA determined that

there were no significant differences between the three groups for any of the three

structure variables across the two-year trial or at any time point during the trial. One-way

ANOVA of AUC values gave the same results with no significant differences being

detected between any of the three groups.

6.4.2.3   Cohort Divided into 6 Groups: 5 Active Therapy Groups and Control

Repeated measures ANOVA suggested that there might be a difference in how Ha

responded to different treatments over the two-year trial. Post-hoc testing indicated that

the ALN and HRT groups were the two treatment groups that were the most different

from each other (p=0.062) with the HRT group increasing in Ha and the ALN group

decreasing in Ha over the trial. One-way ANOVA supported these findings. At 12

months, ALN and HRT were approaching a significant difference in Ha (post-hoc

p=0.088) and at 24 months the difference in Ha of 4.15 mm^2 between the ALN and HRT

groups was significantly different (p<0.05). At the 18-month mark, ANOVA detected a

significant difference in Ha between ALN and A+H groups (post-hoc p<0.01) and a

nearly significant difference between A+H and the control group (post-hoc p=0.056). In

both instances the A+H group had increased more in Ha than the other group. Using

AUC values in the one-way ANOVA found similar trends in Ha at both the 12 and 24-

month time points. At 18-months, however, the AUC method determined that the two

groups that were the most different were the ALN and HRT groups (post-hoc p=0.088).

Neither repeated measures ANOVA, nor one-way ANOVA (using either absolute or

AUC values) detected any differences between the six groups for either the Hm or C.I.

structure variables.

6.5 Discussion

6.5.1 DXA

Percentage BMD losses after 1 and 2 years at the lumbar spine (-0.7% and −1.2%,

respectively) and femoral neck (-0.5% and −0.6%, respectively) in the control group of

this study were similar to those found in placebo or control groups from other

studies[Liberman et al. 1995; The Writing Group for the PEPI Trial 1996; Wimalawansa

1998; Pols et al. 1999; Bone et al. 2000]. For example, after 1 year the PEPI trial detected

a 1.4% loss in lumbar spine BMD in the placebo group that reached 1.8% by 3 years[The

Writing Group for the PEPI Trial 1996]. In the same trial, femoral neck BMD dropped

1.7% in the placebo group by 3 years[The Writing Group for the PEPI Trial 1996]. In the

Phase III alendronate trials, Liberman et al. reported spinal BMD losses of around 0.7%

in the placebo groups and around 1.2% losses at the femoral neck after three

years[Liberman et al. 1995]. Another study testing HRT and etidronate found losses in

their control group of 0.9% at the spine and 2.2% at the femoral neck after two

years[Wimalawansa 1998]. Other studies such as Bone et al. (2000), found losses in their

placebo group (0.6% at both the hip and spine) after 2 years which were similar to the

losses found in our control group, but were also not significant from baseline[Recker et

al. 1999; Bone et al. 2000]. As well, the large FOSIT study of over 1500 women with low bone mass found no significant changes in BMD at the spine or the hip in the control group after one year of the trial[Pols et al. 1999].

The reason that the losses in BMD at the spine and hip in the control group of the present study were not statistically significant could be due to the small sample size of 32 women (with a power of 80% and $\alpha=0.05$, a 2.3% change could have been detected with N=32). One hundred and fourteen subjects would have been needed to establish the 1.2% change in LS BMD at 24 months as statistically significant. It should also be noted, however, that other studies such as the FIT trial have found gains in BMD from baseline at the spine and hip in the placebo group[Black et al. 1996; Cummings et al. 1998]. The FIT trial included calcium and vitamin D supplementation to all subjects, which may explain the gains in BMD found at the spine and hip. Similarly, the calcium and vitamin D supplementation used in the present study could have attenuated bone loss in these subjects so that the percentage BMD losses from baseline were not large enough to be statistically significant in our sample. Overall, the changes in DXA BMD in the control group of this study were in line with the results presented from other osteoporotic drug treatment studies and show that bone at the spine and hip in our control group responded as would be expected.

The group of 91 subjects who were taking any active therapy also responded as would be expected from the results of previous trials studying the effects of anti-resorptive therapy on the skeleton[Liberman et al. 1995; The Writing Group for the PEPI

Trial 1996; Wimalawansa 1998; Pols et al. 1999; Bone et al. 2000]. The present study

found significant increases in BMD in the treatment group at the lumbar spine of 3.8%

and 5.5% and at the femoral neck of 1.5% and 1.6% at one year and two years,

respectively. The PEPI trial found very similar results using HRT with significant gains in

lumbar spine BMD of around 3-5% by 1 and 3 years, and 1.7% at the femoral neck by 3

years[The Writing Group for the PEPI Trial 1996]. Trials involving ETD treatment in

postmenopausal women found BMD gains at lumbar spine of around 2-5% and at the

femoral neck of 0-2% after two and three years of treatment[Watts et al. 1990; Storm et

al. 1990; Wimalawansa 1995; Herd et al. 1997; Wimalawansa 1998; Adami et al. 2000].

BMD gains at the femoral neck in subjects taking etidronate were less with gains of 1.1%

and 1.4% at two and three years[Watts et al. 1990; Harris et al. 1993]. In the large trials

using alendronate (FIT, FOSIT and Phase III trials), increases in lumbar spine BMD in

subjects on treatment were around 4-5% at one year and 5-7% after two years[Liberman

et al. 1995; Black et al. 1996; Cummings et al. 1998; Pols et al. 1999; Bone et al. 2000].

Gains in femoral neck BMD in the treated subjects for these same trials were around 2-

3% at one year and 2.5-3.5% at two years. Subjects in the present trial had similar percent

gains from baseline for both the lumbar spine and femoral neck to those reported in past

trials using HRT and etidronate, and gains detected at the spine were similar to gains

produced by alendronate[Watts et al. 1990; Storm et al. 1990; Harris et al. 1993;

Liberman et al. 1995; The Writing Group for the PEPI Trial 1996; Black et al. 1996;

Cummings et al. 1998; Pols et al. 1999]. Subjects from the large alendronate trials may

have experienced slightly larger gains in femoral neck BMD (2.5-3.5%) after two years,
than those seen in the women on active therapy in this trial (1.6%)[Liberman et al. 1995;
Black et al. 1996; Cummings et al. 1998; Bone et al. 2000]. The group of women who
were taking any active therapy in this trial were prescribed any of 5 different treatment
regimens, which could have reduced or confounded the gains in the subjects taking
alendronate. The separate treatment groups are considered below in the portion of the
discussion dealing with the total cohort divided into 5 active treatments and one control
group. Overall, comparison of BMD changes from baseline for women in the present
study who were taking active therapy to the BMD changes in treated subjects from the
large randomized controlled trials discussed above, shows that our subjects responded to
treatment as would be expected. Treated subjects in the present study also showed greater
gains in BMD at the lumbar spine than at the hip. This heterogeneous pattern of bone
gain, with the spine gaining more than the hip, was also reported in the large randomized
controlled trials, which further supports the contention that subjects in our study
responded to treatment as would be expected[Watts et al. 1990; Harris et al. 1993;
Liberman et al. 1995; The Writing Group for the PEPI Trial 1996; Black et al. 1996;
Cummings et al. 1998; Pols et al. 1999].

Significant treatment effects (differences between treatment and control groups)
were also seen at the hip and spine. Repeated measures ANOVA found significant
treatment effects over the two years for both the spine and hip. T-tests however identified
significant treatment effects only at the spine for both time points but not at the hip for

either time point. The repeated measures ANOVA takes into account the variability of the changes within each group over time, while the t-test method does not. The repeated measures ANOVA is therefore more global which could explain why this method found significant differences at the femoral neck between treatment and control groups over the 2-year trial while the AUC and absolute value t-test methods did not. It should be emphasized, however, that the t-test method at the hip did still show a trend towards the treatment group gaining more bone than the control group (p=0.060-0.077). Other studies have also found significant treatment effects at the spine and hip[Storm et al. 1990; Wimalawansa 1995; The Writing Group for the PEPI Trial 1996; Herd et al. 1997; Wimalawansa 1998; Bone et al. 2000] even in cases where the placebo group gained bone over the course of the trial[Black et al. 1996; Cummings et al. 1998].

Once the group of subjects taking active therapy was divided into those taking one anti-resorptive drug and those taking two drugs, it was found that both the '2-Tx' group and the '1-Tx' group had significant BMD gains from baseline at 12 and 24 months for the lumbar spine. Results also showed that in this sample of postmenopausal women, both the '1-Tx' and '2-Tx' groups gained significantly more bone at the spine than the control group at both the 12 and 24-month time points. Furthermore, the '2-Tx' group gained significantly more spinal BMD at 24 months than the group of subjects taking only one anti-resorptive medication.

When considering the femoral neck, the '2-Tx'group had significant gains at both time points, whereas the '1-Tx' did not have significant gains at either 12 or 24 months

for the hip. Although there were small positive changes from baseline in hip BMD in the '1-Tx' group (0.8% and 0.4% at 12 and 24 months, respectively), this study did not have the power to detect them. The present study had a 0.80 power (α=0.05, two-tailed) to detect a change of 2.3% in the '1-Tx' group at the femoral neck (using the average SD for 12 and 24 months). All else being similar, the number of subjects needed to detect such small changes in the hip, as seen in this study, would be approximately 600 to 1200 women. When groups were compared to each other at the hip, the '1-Tx' and control groups were found to be similar, whereas the '2-Tx' group gained significantly more bone at the hip than either the control or the '1-Tx' group. These results suggest that taking either one or two anti-resorptive treatments increases bone density at the lumbar spine compared to both baseline values and versus a control group taking no anti-resorptive therapy. At the femoral neck, however, there seems to be no significant advantage of taking one active drug treatment over taking no medication. In contrast, subjects on two active therapies tend to increase hip BMD significantly more than either subjects on no treatment or taking one active therapy. These findings suggest that there are skeletal advantages of administering two anti-resorptive therapies at the both the hip and spine over only one anti-resorptive therapy or no active therapy.

Other trials investigating combination therapies have also seen similar results in BMD, showing an advantage of taking combination therapies versus taking a single anti-resorptive drug[Wimalawansa 1995; Wimalawansa 1998; Bone et al. 2000].

Wimalawansa studied the effects of randomly allocated treatments of HRT, ETD and the

combination of these two drugs in 58 early postmenopausal women[Wimalawansa 1995].

It was found that women in the combination therapy group (taking 2 anti-resorptive

treatments) gained significantly more bone at the hip and spine after 2 and 4 years of

therapy than women taking only one anti-resorptive medication. A similar second study

was performed by Wimalawansa in 72 postmenopausal women with low bone mass and 1

to 4 vertebral fractures investigating HRT, etidronate and the combination of these two

drugs[Wimalawansa 1998]. Results supported the previous trial. The women in the

combination therapy group gained significantly more BMD at the hip and spine than

either the etidronate or HRT group at 2 and 4 years, except at the hip at two years. At this

time point the gain in BMD at the femoral neck in the combination therapy group (4.7%)

was significantly greater than that of the etidronate group (1.2%), but not the HRT group

(2.5%). Perhaps the best evidence to date was reported by Bone et al.[Bone et al. 2000].

They performed a two year, double-blinded, placebo-controlled randomized trial in 425

postmenopausal women who had low bone mass and had undergone a hysterectomy.

Treatment groups in this study were HRT, alendronate, HRT plus alendronate and a

placebo group. Results at the lumbar spine were very similar in magnitude to the present

study and showed that the combination therapy group gained significantly more BMD

(8.3%) than either the HRT or alendronate groups (6.0% for both)[Bone et al. 2000].

When comparing results at the femoral neck to the present study, Bone et al. reported

slightly lower BMD gains in the two-treatment group (4.2% versus 6.4% in the present

study) and slightly higher gains in the one-treatment groups (2.6% for HRT and 2.9% for

alendronate groups versus 0.4% in the '1-Tx' group of the present study). However, in accordance with the findings of the present study, Bone et al. still found that subjects taking combination therapy gained significantly more BMD at the femoral neck than either the HRT or alendronate groups alone[Bone et al. 2000].

After the total cohort was divided into the five separate active therapy groups and the control group, each active treatment group was found to have gained a significant amount of lumbar spine BMD from baseline. Furthermore, repeated measures ANOVA with post-hoc testing determined that each of the separate active treatment groups produced a significant treatment effect (i.e. gained significantly more lumbar spine BMD than the control group) over the two-year trial. These results show that each of the anti-resorptive therapies had positive bone-gaining effects at the lumbar spine. As individual treatments, each of these anti-resorptive therapies showed similar results to those found in previous trials[Watts et al. 1990; Storm et al. 1990; Liberman et al. 1995; Wimalawansa 1995; The Writing Group for the PEPI Trial 1996; Black et al. 1996; Cummings et al. 1998; Wimalawansa 1998; Recker et al. 1999; Bone et al. 2000]. These findings maintain that the subjects in this study responded to the various individual drug treatments as would be expected at the lumbar spine. With repeated measures ANOVA the A+H group showed a trend for a greater gain in spine BMD than the ETD group (p=0.051) and one-way ANOVA at 24 months showed that the A+H group had a greater percent gain than the HRT or ETD group, which further supports the notion that combination therapy may be more beneficial than a single active drug treatment. Graphing of the percent change in

lumbar spine BMD visually depicts the trend of effectiveness for each of the therapies, which is even more evident by 24 months (see FIGURE 6.3 in section 6.2.1.2). There seems to be a trend for the two combination therapy groups to have greater gains in BMD, followed by ALN, HRT and finally ETD. The control group is the only group that had a negative change in spinal BMD from baseline. This decline in lumbar spine BMD in the control group did not reach significance at either time point, but was approaching significance at 24 months with a decrease of 1.2% (p=0.062).

The effect of the individual treatment regimens on BMD at the femoral neck was less straightforward. Only the A+H and ALN groups saw significant gains in hip BMD at 12 months. Even though the E+H group gained more bone than the ALN group at 12 months, this increase did not reach statistical significance, likely due to the small number of subjects in the E+H group and the large variance. Similar to the 12-month mark, the E+H group had a relatively large gain in bone density at 24 months (4.5% gain), but this did not reach significance likely due to the small sample size and especially the large variance (SD=7.1). A sample size of 41 women would be needed to detect the 4.5% increase in FN BMD with a SD=7.1 (power=0.80 and α=0.05). If the variance in this group was closer to that seen in the other treatment groups (i.e. SD=5), then a sample size of only 21 would be needed to detect a 4.5% change in hip BMD from baseline (power=0.80, α=0.05). At 24 months the A+H group had a large, highly significant increase in hip BMD (8.4%, p=0.001) that was double the BMD gains seen in two previous larger trials investigating the combined therapy of alendronate and HRT[Tiras et

al. 2000; Bone et al. 2000]. A possible explanation for the large percentage increase in BMD in the A+H group could be that these women, out of all the individual treatment groups in the present study, had the lowest baseline femoral neck T-score. This means that similar absolute gains in FN BMD would appear larger as a percentage gain, than in women who had higher baseline hip BMD values. Tiras et al. and Bone et al. did not report baseline FN T-scores and therefore values could not be compared to the present study.

When comparing hip BMD changes among different groups in the present study by repeated measures ANOVA, the E+H group gained significantly more BMD than the control group at 2-years. Comparing the A+H group to the other treatment groups over the 2-year trial, found that the A+H group gained significantly more bone than ALN, HRT, ETD and the control group. These results suggest that either combination therapy (A+H or E+H) is more effective than no medication for increasing bone density at the hip and that the combination of alendronate and HRT is a more effective treatment than any of the single anti-resorptive therapies for gaining BMD at the femoral neck.

Most treatment groups in the present study, independent of how they were defined, showed greater bone mineral density gains in the lumbar spine than in the femoral neck. This trend of subjects on anti-resorptive therapy gaining more bone at the spine than at the hip is echoed in numerous previous trials studying the effects of single or combination therapy with anti-resorptive agents. [Watts et al. 1990; Harris et al. 1993; Liberman et al. 1995; Wimalawansa 1995; The Writing Group for the PEPI Trial 1996; Black et al. 1996;

Cummings et al. 1998; Wimalawansa 1998; Pols et al. 1999; Bone et al. 2000]. The difference between BMD gains at the spine and hip may partially be explained by the spine having a higher ratio of trabecular bone than the hip[Mundy 1999]. Trabecular bone has a greater surface area per volume than cortical bone, and therefore a greater proportion of bone undergoing bone turnover at any one time. The spine, therefore, would likely be more affected by changes in bone turnover induced by anti-resorptive medications than the hip, since the remodeling space would be greater at the spine.

In summary, subjects taking any anti-resorptive treatment gained more bone at the hip and spine than subjects who did not take any treatment. Single and combined anti-resorptive treatments increased bone density at the spine, but only the combination therapy groups, especially A+H, showed BMD gains at the femoral neck. In general, the combination therapy groups tended to gain the most bone, followed by the single anti-resorptive therapy groups, while BMD in the control group remained relatively stable or declined slightly. There was a noticeable trend suggesting that at the spine and hip, A+H was the most effective therapy, followed by E+H and then the single therapies. ALN appeared to be the most effective single therapy for gaining bone, followed by HRT and finally ETD. Taking no drug treatment was the least effective at producing BMD gains since this group showed trends for bone loss at both the femoral neck and lumbar spine.

6.5.2 PQCT

There have been some studies by different research groups using pQCT technology to measure bone changes at the distal radius with age in postmenopausal women. One

group found that volumetric bone mineral density decreases in the trabecular

compartment but remains relatively constant in the cortex in both a one-year[Ruegsegger,

Durand, and Dambacher 1991A] and a two-year longitudinal study[Ruegsegger, Durand,

and Dambacher 1991B]. Early postmenopausal women lost 2.8% of trabecular density per

year in both studies, while older women with osteoporosis (defined by ≥ 1 vertebral

fracture) lost 1.2% over the one-year trial. Both studies found non-significant changes in

cortical bone density of 0-0.2% per year. These trials used a high-resolution (0.2mm)

Densiscan system and evaluated the inner 50% core as trabecular bone at the ultra-distal

site (ten - 1mm slices, 1.5mm apart), while cortical bone was measured in a special region

of interest inside the cortical radial shaft (six - 1mm slices, 1.5mm apart). This cortical

bone region of interest was completely contained within the cortex, removing any partial

volume effect, which occurs in the voxels along the bone edges. A third study, by

Tsurusaki et al. in Japan, employed this same Densiscan system and analysis for

trabecular bone, but used the entire 100% volume at the diaphyseal site as their cortical

bone region of interest[Tsurusaki, Ito, and Hayashi 2000]. This trial found similar results

to the previous two studies for trabecular loss at the ultradistal site in postmenopausal

women (-2.3%/year), but larger losses for cortical bone (-1.2%/year). Different pQCT

systems (manufactured by Stratec) define cortical bone regions of interest that are not

contained entirely within the cortical shell. Such devices find comparable results for

cortical bone loss with age to those reported by Tsurusaki et al.(2000). For example,

cortical density losses for the diaphyseal radial site in postmenopausal women ranged

from -0.2% to −1.4% per year while bone losses measured at the ultradistal radius were similar, or slightly larger, ranging from -0.5% to -2.3% loss per year[Gatti et al. 1996; Boonen et al. 1997; Hernandez et al. 1997; Nijs et al. 1998; MacIntyre, Adachi, and Webber 1999; Martin and Reid 1999]. Yearly losses in trabecular density at the ultradistal radius in postmenopausal women have been reported to range from -0.4% to -2.7%[Gatti et al. 1996; Boonen et al. 1997; Hernandez et al. 1997; Nijs et al. 1998; Schneider et al. 1999; Martin and Reid 1999; Guglielmi et al. 2000]. In general, studies reporting heterogeneity in yearly losses of trabecular and cortical bone density with age, have seen greater rates of loss in the trabecular compartment than the cortical compartment[Ruegsegger, Durand, and Dambacher 1991A; Ruegsegger, Durand, and Dambacher 1991B; Boonen et al. 1997; Nijs et al. 1998; Tsurusaki, Ito, and Hayashi 2000]. One recent study, however, measured density changes in the trabecular, cortical and subcortical bone compartments and found slightly greater losses in the cortical and subcortical envelopes (-0.5 to −1.4% and -0.7 to -1.4% per year, respectively) than in the inner 45% trabecular core (-0.5 to −0.8% per year)[Martin and Reid 1999]

In the present study, the control group showed a borderline significant loss of nearly 2% (p=0.052) in TOT-vBD by two years and a trend of TRAB-vBD loss (-1.3%, p=0.157). Cortical density remained relatively constant across the two-year trial. The trend in these results, which suggest a greater loss in trabecular than cortical density, is similar to that seen in the majority of previous studies[Ruegsegger, Durand, and Dambacher 1991A; Ruegsegger, Durand, and Dambacher 1991B; Boonen et al. 1997;

Nijs et al. 1998; Tsurusaki, Ito, and Hayashi 2000]. The rate of loss of TOT-vBD of

around 0.8% per year in the present study is similar to values reported in other

longitudinal and cross-sectional pQCT studies that have shown losses in total density at

the distal radius of 0.6 to 2.3% per year in postmenopausal women[Ruegsegger, Durand,

and Dambacher 1991A; Butz et al. 1994; Gatti et al. 1996; Hernandez et al. 1997; Nijs et

al. 1998; Schneider et al. 1999; MacIntyre, Adachi, and Webber 1999; Martin and Reid

1999; Tsurusaki, Ito, and Hayashi 2000; Guglielmi et al. 2000]. Since cortical density was

stable over the two years and trabecular density decreased, then the loss in total bone

density was likely due to changes in the trabecular compartment. The findings in this

study that trabecular bone density decreased faster than cortical bone density and that the

rate of total bone density loss was similar to other pQCT studies support the contention

that the women recruited for the present study show expected bone changes with age.

In the present study trabecular bone was separated from cortical bone by an

operator-independent method that places the boundary between the two compartments

along the contour of greatest change in density. For example, if density were graphed

from the center of the bone to the outer edge for a line along each radius, then the

separating contour would be placed at the site of the greatest slope for each line. Age or

treatment can cause bone changes at the endocortical surface of the radius. Consequently,

the boundary between cortical and trabecular bone may also change along with the

respective cross-sectional areas. It follows that the bone density results should not be

regarded in isolation, but should be considered together with the cross-sectional area and

bone content measures. Although cortical density was found to be relatively stable over the two years, there were significant losses in cortical content detected at 12 and 24 months in the control group (-2.1% and –2.3%, respectively, both p<0.05). Along with the loss of CRT-CNT, there was a trend for a loss in CRT-CSA. The cortical area was slightly lower than baseline at all time points, with the magnitude of loss peaking at – 2.5% (p=0.068) at 12 months. These findings of a stable cortical density, a significantly decreased cortical content and a trend for a similar decrease in cortical cross-sectional area, suggest that women in the control group experienced cortical thinning (likely due to endocortical resorption) with little increase in intracortical porosity at the distal radius over the two year trial. In the trabecular compartment there were no significant changes detected from baseline. The trends show a loss in trabecular density and a gain in cross-sectional area while trabecular content remains virtually unchanged. The enlargement in trabecular cross-sectional area is expected and supports the suggestion that endocortical resorption likely occurred in the control group. As the area increases, trabecular bone content would be predicted to increase as well, but this did not occur in the control group. Trabecular thinning and potentially complete loss of trabecular elements would explain that while TRAB-CSA enlarges there is both a consistent pattern of a small loss in TRAB-vBD and no change in TRAB-CNT. Previous cross-sectional studies have also found evidence for endocortical resorption at the ultradistal radius in women with age[Gatti et al. 1996; Nijs et al. 1998]. Gatti et al. measured the non-dominant radius with pQCT in 29 premenopausal and 241 postmenopausal women and found significant

increases in both the total and trabecular bone areas while cortical area stayed relatively

constant with increasing age[Gatti et al. 1996]. In this same study, cortical bone area at

the proximal radius was found to decrease with age. A second cross-sectional study using

pQCT, measured 275 postmenopausal women and also found increases in trabecular and

total bone areas with age but did not report changes in cortical area[Nijs et al. 1998].

These two studies support the idea that endocortical resorption occurs with age since the

trabecular area was found to increase with increasing age.

In contrast to the present study, however, the two cross-sectional studies detected

increases in total bone area, suggesting periosteal accumulation of bone with age[Gatti et

al. 1996; Nijs et al. 1998]. Total cross-sectional area in the present study did not differ

significantly from baseline at any time point. One reason for this difference could be that

subjects in both of the cross-sectional studies spanned a large age range. It would likely

be easier to detect differences in bone area between groups of women who are 30-40

years apart in age than to detect changes in total bone area over only two years, as in the

subjects followed in the present study. These subjects would likely have to be followed

for a longer duration in order to observe changes in the periosteal surface and total bone

area. Total bone content, on the other hand, was significantly lower than baseline at all

time points in the control group and decreased by 2% ($p < 0.01$) at two years. This shows

that the subjects in the control group lost overall radial bone mass with age. In summary,

it is proposed that endocortical resorption and trabecular thinning are mechanisms that

can explain the changes in distal radial bone mass, density and area that are found in the control group in this two-year trial.

The 'Any-Tx' group showed significant gains in total and cortical volumetric bone density, but significant losses in trabecular bone density at the distal radius. The cross-sectional area and bone content of the trabecular compartment were also found to decrease with anti-resorptive therapy. These changes in the trabecular envelope are consistent with endocortical apposition. As bone is accumulated at the trabeculo-cortical junction, the pQCT analysis will include more bone in the cortical compartment and less bone in the trabecular compartment. The trabecular compartment will not only become smaller, but also include a higher proportion of the innermost trabeculae. These inner trabeculae are likely thinner and more perforated than the outer trabeculae, which accounts for the decrease in TRAB-vBD. Cortical bone content increases, due to either endocortical accumulation or a decrease in intracortical porosity (increase in cortical density by filling of Haversian systems) or a combination of both these mechanisms. Since cortical cross-sectional area does not increase, and in fact shows a small decrease, the more likely mechanism for an increase in cortical content is a reduction in intracortical porosity. The small decrease in cortical area does not challenge the proposed therapeutic mechanism of endocortical apposition because total area also decreases with treatment. This means that the endocortical surface can be gaining bone with treatment, but at a slower rate than the periosteal surface is losing bone. The gain in total bone density can be explained by the suggested mechanisms of endocortical accumulation and

a reduction in intracortical porosity. What seems to be confusing is that total bone content and cross-sectional area both decreased with anti-resorptive therapy. For there to be a decrease in total bone content while bone is being added to the endocortical surface and intracortical canals, then bone must be lost from somewhere else. The two possible sites are the periosteal surface and the inner trabecular network. Since total cross-sectional area decreases with treatment, then this supports periosteal resorption. Trabecular thinning is a second possible mechanism, which could also explain the loss in trabecular density seen with anti-resorptive therapy. The question now becomes why. What would cause trabecular thinning and/or periosteal resorption in the group of women receiving treatment? With endocortical and intracortical bone accumulation, the cortex becomes stronger and stiffer, which effectively unloads the central trabecular bone and perhaps also the outer periosteal bone. As this alteration in loading is perceived, bone cells in their respective 'unloaded' areas signal for resorption to commence. Another hypothesis could be that anti-resorptive therapy reduces bone turnover, which helps fill in the remodeling space (volume of bone that has been resorbed, but not yet replaced), but is unable to overcome a significant negative bone balance (more bone resorbed than replaced per remodeling unit)[Seeman 2002]. If this is the case, then bone may still be lost from the trabecular or periosteal compartments if these sites have a large negative bone balance. In summary, it is proposed that endocortical and intracortical accumulation are the primary mechanisms that can explain the changes in distal radial bone mass, density and area that are found in the 'Any-Tx' group in this two-year trial. The accompanying unloading of the

trabecular and periosteal compartments or large negative bone balances lead to bone loss

in these sites through the secondary mechanisms of periosteal and trabecular resorption.

The only identified previous study using pQCT at the distal radius to follow anti-

resorptive therapy (specifically, alendronate) in postmenopausal women was the FOSIT

sub-study of Schneider et al.[Schneider et al. 1999]. In the sub-study only total and

trabecular density were measured; neither cortical density nor any measurements of bone

content or cross-sectional area were reported. Schneider et al. found similar results to the

present clinical trial with a significant gain in TOT-vBD in the alendronate-treated group

and trends for losses in TOT-vBD and TRAB-vBD in the control group. Although

Schneider et al. found no significant change in TRAB-vBD in the treatment group, they

reported a trend for a gain in trabecular density (+5.3%, CI_{95} -4.26 to 14.83), which is

different from the significant loss of 2.3% found in the present study. However, in

contrast to the method used for definition of the trabecular/cortical junction in this study,

Schneider et al. used a fixed value corresponding to the inner 45% of the bone cross-

sectional area as the trabecular compartment. Their results suggest that trabecular

thinning does not occur with treatment. In fact, the trend in their data suggests that there

may be an increase in trabecular bone mineral with alendronate. It must be kept in mind

that Schneider et al. only studied alendronate whereas the present study included five

different treatment regimens. Still, the trend in the data from Schneider et al. would

support the hypothesis that the increase in cortical bone density found in the present study

unloads the outer bone more than the inner bone and total bone content would likely be

lost from the periosteal surface with treatment, rather than from the inner trabeculae. The results from Schneider et al. also support the theory that the loss of trabecular density found with treatment in the present study is not due to trabecular thinning, but due to a larger proportion of the trabecular compartment being composed of the smaller inner trabeculae due to endosteal apposition and the subsequent shift of the trabecular boundary inwards.

When the women in the present study were divided into '1-Tx', '2-Tx' and control groups, women on combination therapy gained more bone density and mass in the cortical compartment than women on no treatment. Women in the '2-Tx' group also showed trends for greater gains in cortical mass and density than the women in the '1-Tx' group. Furthermore, the combination therapy group showed trends for losing less bone mass and density in the trabecular compartment than the single treatment group. The effects of combination therapy in the cortical and trabecular compartments are both in the direction of slowing the loss of or increasing total bone content and density. The '2-Tx' group gained significantly more total bone content than the '1-Tx' group. Total bone density gains at the distal radius were also significantly greater in the '2-Tx' group than the control group and showed a trend for greater gains than the '1-Tx' group. In summary, these results suggest that combination therapy increases total bone density and content at the distal radius more than single therapy by producing greater gains in the cortical compartment while reducing the treatment-associated bone loss in the trabecular compartment.

When the women in the present study were divided into all five treatment groups

and a control group there were no differences found between the 5 different active

therapy groups by repeated measures ANOVA. Individual treatments were, however,

found to have significantly different effects on radial bone than in the control group for

the cortical compartment and whole bone, but not for trabecular bone. These differences

were primarily found between one of the combination therapy groups (A+H or E+H) and

the control group, except for cortical content, where both the combination therapy groups

and the HRT group were all found to have gained more bone than the control group using

repeated measures ANOVA. These gains in cortical bone mass led to significant or

borderline significant gains over the control group for A+H in total bone mass (p=0.038)

and for E+H in total bone density (p=0.057). The HRT group had no significant gains in

total bone mass or density, likely due to the noticeable and consistent trends for bone loss

in the trabecular compartment. For example, the HRT group showed a trend for a greater

loss in trabecular bone content than the control group with repeated measures ANOVA

(Dunnett's t-test, p=0.094).

6.5.3 Trabecular Structure

During the course of the trial, the structural changes observed in the control and

treatment groups were subtle but the data show trends consistent with the following

hypotheses. Based on pQCT density, content and geometry measures, women in the

control group experienced endocortical resorption and trabecular thinning. Endocortical

resorption would likely produce no change in Hm, which will probably be a measure of

the diameter of a relatively large central marrow pore. Ha, however, would likely decrease as new pores developing at the inner surface of the cortical shell (trabecularization at the endocortical envelope) would be smaller than the preexisting central trabecular pores. CI is likely to increase following the same reasoning. Trabecular thinning (with occasional perforation or loss of trabecular elements) would be expected to produce an increase in both Ha and Hm and a decrease in connectivity. If the effects of these two mechanisms were equivalent one would anticipate no change in Ha, no change or a small increase in Hm and no change in CI. The trends in structure indices of the women in the control group in this study follow these predicted patterns. The *in vivo* measurement of trabecular architecture used in this study, therefore, seem consistent with the expected trends at the distal radius with aging. To detect these perceived trends as statistically significant changes, however, would require a prolonged study with larger groups of subjects due to the small, slow changes in structure indices and the large population standard deviations. The data suggest that Hm may be the most sensitive of the trabecular structure indices to identify change over time at the distal radius in untreated postmenopausal women with low bone mass.

Trabecular structure indices in the 'Any-Tx' group also behaved in a manner consistent with the proposed mechanisms based on bone density, content and geometry changes at the distal radius due to anti-resorptive therapy. The mechanisms include decreased cortical porosity, endocortical accumulation and potentially, trabecular thinning. Changes in cortical porosity would not affect trabecular architecture directly but

may indirectly alter structure through increases or decreases in loading of trabecular struts. A decrease in cortical porosity (increase in CRT-vBD) with a concomitant increase in stiffness of the cortical shell would likely unload some trabeculae and perhaps promote trabecular thinning[Ferretti 1997]. Trabecular thinning would cause an increase in Ha and potentially Hm, as well as a decrease in connectivity. Endocortical accumulation would likely decrease connectivity, not affect Hm and would probably increase Ha as some of the smaller pores are filled and therefore removed from the computed average. If the effects of endocortical accumulation and trabecular thinning were equivalent, then this would predict an increase or no change in Ha, a small increase or no change in Hm and a decrease in connectivity. In the 'Any-Tx' group of this study, Hm and Ha remained constant over the two years, while connectivity significantly decreased (p=0.009 at 18 months). Similar to the control group, *in vivo* measurement of trabecular architecture in the 'Any-Tx' group of this study identifies the expected trends in structure changes at the distal radius with anti-resorptive therapy. The data suggest that CI may be the most sensitive of the trabecular structure indices to detect change over time at the distal radius in postmenopausal women with low bone mass who are receiving anti-resorptive therapy.

The trabecular structure indices in this trial had poorer reproducibility than the radial density, mass and content measures by pQCT. This may have compromised the ability to detect significant changes in trabecular architecture. Even with these limitations, analyses were re-run with the treatment study population divided into 3 groups ('1-Tx', '2-Tx' and control) and again after separation into six groups (five different active treatments and

control) protocols. There were few statistically significant changes from baseline and no differences were detected between groups at any time over the two-year trial for both the 3-group and 6-group analyses. There was, however, a noticeable trend when visually inspecting the graphed results of the cohort divided into the five active therapy groups and a control group. The trend was that HRT seemed to have the greatest treatment effects on structure indices while ALN had the smallest effects. HRT had the largest decrease in CI (-2.0 to -2.5), which reached statistical significance at 6, 12 and 18 months (p=0.034, 0.039 and 0.036, respectively) and approached significance at 24 months (p=0.068). HRT also showed a trend for producing the largest gains in Hm and Ha for any of the treatment groups, with a change in Ha at 18 months that was approaching significance (p=0.077). ALN was consistently found to affect structure the least or in the opposite direction than HRT. For example, ALN showed a trend for a decrease in Ha at all time points with the decrease becoming borderline significant at 24 months (p=0.061). It is also interesting to note that these same trends for HRT and ALN are not generally found in the pQCT measurements of mass, content and area, except in the trabecular compartment. The trabecular compartment consistently shows a trend for greater treatment effects with HRT than any of the other treatment groups (Dunnett's post-hoc test for repeated measures ANOVA for TRAB-CNT found p=0.094 for HRT versus control). These trends in the effects of different treatments on the trabecular compartment and trabecular structure suggest that HRT may have a different mechanism of action than alendronate, or a more potent treatment effect, specifically in trabecular bone.

CHAPTER 7: PREDICTING THE FAILURE LOAD OF THE DISTAL RADIUS

7.1 Abstract

The distal radius is an important site for the early detection of patients at risk for fracture. Since measuring bone strength *in vivo* is not possible, we evaluated which bone assessment method of the forearm would best predict failure load of the distal radius and computed a factor of risk for wrist fracture (Φ_{wrist}). Thirty-eight cadaveric forearm specimens were measured by five different techniques to assess bone density, bone mineral content, geometry and trabecular structure at the distal forearm. The bone assessment techniques included dual-energy X-ray absorptiometry (DXA) of the radius, peripheral quantitative computed tomography (pQCT) of the 4% and 20% distal sites of the radius, DXA of the phalanges, digital x-ray radiogrammetry of the forearm (DXR-BMD), and quantitative ultrasound of the radius. The failure load of each excised radius was determined by simulating a fall on an outstretched hand. The pQCT measurements of polar stress-strain index and cortical content explained the greatest portion of variance in failure load ($r^2 = 0.82 - 0.85$). Bone mineral content measures were generally better predictors of failure load ($r^2 = 0.53 - 0.85$) than the corresponding volumetric or areal bone mineral density values ($r^2 = 0.22 - 0.69$) measured by either pQCT or DXA. Multiple regression analysis showed that the addition of a bone geometry measure

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improved the ability of a bone density measure alone to predict failure load. There was

high variability in the ability of different techniques and different variables within a given

technique to predict failure load. Estimates of the factor of risk for wrist fracture (Φ_{wrist})

revealed that the women in this study would have been likely to fracture their distal radius

upon falling from a standing height ($\Phi_{wrist} = 1.04$), whereas the men would have likely

withstood the impact without fracturing their wrist ($\Phi_{wrist} = 0.79$).

Keywords: Bone mineral density; Bone strength; Dual X-ray Absorptiometry; Peripheral

Quantitative Computed Tomography; Radius; Structure

7.2 Introduction

Osteoporosis is defined as 'a skeletal disorder characterized by compromised bone

strength predisposing to an increased risk of fracture' specifically at the hip, spine and the

distal radius [Consensus Development Conference 2000]. Fractures of the distal radius,

also known as Colles' fractures, are the most common fractures in women less than 75

years of age [Owen et al. 1982]. The incidence of Colles' fracture peaks at age 65, well

before the maximum incidence for spine and hip fractures [Eastell 1996]. Because these

wrist fractures occur approximately 10 to 15 years before hip and spine fractures, they

may be important, early indicators of future fracture risk. Indeed, several studies have

shown that a positive history for Colles' fracture is a predictor of future fractures of all

types [Gardsell et al. 1993; Mallmin et al. 1993; Cuddihy et al. 1999]. Specifically, the

presence of a Colles' fracture substantially increases an individual's risk for both hip (1.4

fold in women, 2.7 fold in men) and vertebral fractures (5.2 fold in women, 10.7 fold in

men) [Cuddihy et al. 1999]. Taken together, these observations indicate that the distal

radius could play an important role in the early detection of individuals at risk for

osteoporotic fracture.

In general, fractures occur when the force on a bone exceeds the maximum load that

it can bear. For obvious reasons, the failure load of a bone cannot be directly measured *in*

vivo. Therefore, other characteristics that are surrogates of bone strength must be

measured non-invasively. These *in vivo* estimates of radial bone strength, along with

other factors such as propensity to fall and magnitude of trauma, help to predict the risk

for a wrist fracture.

Currently, osteoporosis and fracture risk are most commonly assessed by measuring

areal bone mineral density (BMD) with dual-energy X-ray absorptiometry (DXA). BMD

is a strong predictor of future fracture risk [Marshall, Johnell, and Wedel 1996].

However, when fracture and non-fracture patients are compared, there is a notable

overlap in BMD values between the two groups [Melton, Eddy, and Johnston 1990;

Greenspan et al. 1994]. Therefore, it is likely that there are other factors besides BMD

that affect whether an individual will suffer a fracture. These other factors may include

frequency and severity of falls [Greenspan et al. 1994], as well as factors directly

affecting bone strength, such as a less than optimal bone geometry or trabecular structure

[Gordon, Webber, and Nicholson 1998], or perhaps an accumulation of

microdamage[Burr et al. 1997]. Thus, it may be that combining a BMD measurement

with other bone characteristics will improve fracture risk assessment.

Therefore, considering the possibility that the distal radius may be useful as an early indicator of future fracture risk, the primary goal of this study was to evaluate several bone assessment techniques to identify the bone variable or combination of variables that best predicts failure load at the distal radius. In addition, we assessed gender-related differences in mechanical and densitometric properties of the distal radius, and computed the 'factor of risk' (Φ_{wrist}) for wrist fractures[Hayes, Piazza, and Zysset 1991].

7.3   Methods

7.3.1   Cadaver Specimens

Thirty-eight left forearms were obtained from the Anatomical Gifts Program at Harvard Medical School. The sample included 18 female and 20 male donors with a mean ($\pm$SD) age of 78 $\pm$ 12 years (range: 53-97 years) and mean weight of 64 $\pm$ 15 kg (range: 40-93 kg). The forearm specimens were harvested using a single transverse cut at the mid-humerus. Specimens were obtained fresh with all soft tissues intact, and were stored frozen until testing. Radiographs of each specimen were acquired and screened to eliminate specimens with evidence of metastatic bone tumor or previous fracture. The length of each forearm was measured from olecranon process to ulnar styloid.

7.3.2   Bone Assessment Techniques

Several different techniques were used to assess skeletal status of the intact cadaveric forearms: 1) DXA of the distal radius, 2) DXA of the phalanges, 3) peripheral quantitative computed tomography (pQCT) of the distal radius, 4) quantitative ultrasound

(QUS) at the distal radius, and 5) digital x-ray radiogrammetry (DXR) of the forearm and hand.

DXA (QDR2000+, Hologic, Bedford, MA, USA) was used to assess bone mineral content (BMC, g) and areal bone mineral density (BMD, g/cm^2) of the radius at the ultradistal (UD) and one-third distal regions (FIGURE 7.1). A T-score was also calculated for each specimen at the UD and one-third distal regions. Scans were acquired and analyzed according to standard procedures defined by the manufacturer. DXA (AccuDXA, Schick Technologies, Long Island City, NY, USA) was also used to assess mean BMD of the middle phalanges of the of the 2nd and 4th digits (phBMD, g/cm^2).

PQCT (XCT-960A, Norland Corporation, Fort Atkinson, WI, USA) was used to assess volumetric bone density (vBD, mg/cm^3), mineral content (CNT, mg) and geometric variables in the radius at the 4% and 20% distal sites (FIGURE 7.1). Geometric measurements included cross-sectional area (CSA, mm^2) and moment of inertia of the cortical shell (Ix, mm^4) at both the 4% and 20% sites. The stress-strain index of the cortical shell (SSI-p, mm^3) was also measured at the 4% site (SSI-p = polar moment of resistance multiplied by the ratio of measured cortical density to physiologic bone density ($1200mg/cm^3$)). Due to operator error SSI-p was not available at the 20% site. Measurements at the highly trabecular 4% distal site were made for the trabecular (TRAB), cortical (CRT) and total (TOT) bone compartments. In comparison, at the 20% distal site, only cortical bone measurements were acquired. The pQCT slice thickness was 2.5mm, with in-plane pixel size of 0.59 mm per side and a matrix size of 254 x 254

pixels. High-resolution software (xmice, v1.0, Norland Medical Systems, Inc.) with an in-plane pixel size of 0.33 mm was also used at the 4% site to acquire images for trabecular structure analysis [Gordon et al. 1996]. The trabecular structure analysis was performed using specialized software developed in our laboratory to measure the fraction of bone occupied by marrow (marrow fraction = marrow CSA / marrow CSA + trabecular bone CSA), maximum hole size (Hmax, cm^2) and connectivity (connectivity index, C.I.).

QUS (Omnisense, Sunlight Technologies, Rehovot, Israel) was used to measure the speed of sound (SOS, m/s) along the distal radius using the standard radial probe [Hans et al. 1999]. Before performing the measurements, the forearm specimens were warmed to 37°C in a water bath. The mean of two measurements was used for the analysis. Finally, DXR (Pronosco, Vedbaek, Denmark) was used to compute an estimated BMD for the distal forearm (DXR-BMD) [Jorgensen et al. 2000; Bouxsein et al. 2002]. Standard antero-posterior radiographs of each specimen were obtained using high-resolution mammography film (x-ray settings at 50 kv and 4-5 mAs). Specifically, the cortical thickness at five regions of interest (the radius, ulna, and middle three metacarpals) was measured and used in the calculation of DXR-BMD [Jorgensen et al. 2000].

7.3.3 Mechanical Testing

The mechanical testing configuration was designed to simulate a fall on an outstretched hand (FIGURE 7.2). Radii were excised from the cadaver specimens and cut 7.6 cm proximal to the 25% distal site. Both ends of the radius were embedded in polymethylmethacrylate (PMMA) within square aluminum containers (7.6 cm x 7.6 cm).

The proximal end of the radius was potted to the 25% distal site while the distal end was embedded shallowly to a depth of approximately 0.6 cm. The radii were positioned in 15° of dorsal inclination from the vertical axis as previously described [Myers et al. 1991; Myers et al. 1993; Augat, Reeb, and Claes 1996; Augat et al. 1998]. To simulate impact, a compressive load was applied at a constant displacement rate of 100 mm/s using a servohydraulic materials testing system (Model #1330, Instron Corp., Canton, MA, USA). Load and displacement data were recorded at 1000 Hz and the failure load (N) of the radius was determined from the load-displacement curve. A few specimens had load-displacement curves that did not demonstrate a distinct failure point and were excluded from analysis. An example of a standard load-displacement curve and an irregular curve with no distinct failure point are shown in FIGURE 7.3.

Conventional fracture grading systems, although preferable, could not be used since the aluminum containers at each end of the radius prohibited reliable post-testing radiographs. Instead, the location of the fracture was assessed visually. Since our study focussed on failure load at the distal portion of the radius, where Colles' fractures occur, only those specimens that fractured through the most-distal 10% of the radius were included in subsequent analyses.

7.3.4 Calculation of the Factor of Risk for Wrist Fractures

To investigate the influence of skeletal loading in the assessment of fracture risk, we computed a factor of risk (Φ_{wrist}) for wrist fractures. The factor of risk, first introduced by Hayes et al [Hayes, Piazza, and Zysset 1991], is defined as the applied load / failure load.

For any given bone, when the factor of risk is greater than one, a fracture is predicted to occur. In the present study, the numerator of the factor of risk, (i.e. the applied load) was defined as the load applied to the outstretched hand during a fall from standing height [Chiu and Robinovitch 1998]. This load was estimated as the product of a damping constant (670 Ns/m) and the impact velocity associated with the fall [Chiu and Robinovitch 1998]. The impact velocity was computed as (sqrt(2*9.81*fall height)) where fall height in meters was estimated as 50% of donor height. The denominator of the factor of risk (i.e. the failure load, in Newtons) was taken from the results of the mechanical testing.

7.3.5 Data Analysis Methods

Unpaired t-tests were used to detect any differences between the final sample analyzed and those specimens that were excluded. The final sample was characterized by standard descriptive statistics. Unpaired t-tests were used to assess the effect of gender. Bivariate regression analysis was performed to determine the relationship between individual bone variables and failure load. To evaluate whether the correlation coefficient of one bone variable in predicting failure load was significantly greater than that of another bone variable, the Fisher Z-transform and the z-test were used. Finally, stepwise multiple regression was used to determine whether a combination of variables predicts failure load better than a single variable alone.

The various bone measurements were divided into three groups based on the type of characteristic that was assessed (bone mineral (i.e. density or content), bone geometry or

trabecular bone structure). A combination of bone variables, each measuring a different characteristic, was then entered into a stepwise multiple regression model (α-to-enter = 0.15, α-to-remove = 0.15). We used a combination of three variables, one that reflected total bone mineral (density or content) at the ultradistal region, plus a second variable measuring cortical bone geometry at the 20% site, plus the third variable which was an index of trabecular bone structure at the 4% site. All analyses were considered significant at $p<0.05$ and were performed using commercial statistical software (Minitab release 13, State College, PA, USA).

7.4 Results

Twenty-one specimens were included in the final analysis, nine female and twelve male. Specimens were excluded for the following reasons: previous fracture (n=4), unacceptable mechanical testing (clamp slipped, n=2), fracture location not at the distal radius region (n=4), pQCT software unable to distinguish trabecular and cortical bone at 4% site (n=3) and indistinct failure point on the load-displacement curve (n=4). Donor age and height were similar for the included and excluded specimens, while the excluded donors were 19% lower in weight ($p<0.05$). DXR-BMD and all pQCT measures of bone content and CSA at the 4% and 20% sites, as well as cortical bone density at the 20% site, were similar for the included and excluded specimens. Bone density measurements, however were lower in the excluded specimens. Volumetric bone density by pQCT was 28-32% ($p<0.05$) lower at the 4% site and UD-BMD by DXA was 22% lower in the excluded specimens ($p<0.05$). UD-BMC and phBMD showed trends toward lower values

in the excluded forearms (p = 0.051 and 0.054, respectively). There was also a small, but significant difference in SOS, with the excluded specimens being 3% (p<0.05) lower than included specimens.

7.4.1 Gender-Related Effects

Age and weight were similar for the male and female donors, whereas male donors were 9 cm taller (p<0.05) (TABLE 7.1). Male forearms were larger than the female forearms. For example, TOT-CSA at the 4% site was 34% greater in males than females (p<0.01) and CRT-CSA at the 20% site was 33% greater in males than in females (p<0.001). Consistent with a larger bone size, bone mineral content (assessed by either DXA or pQCT) was 38-74% greater in male specimens (p<0.05). In addition, phBMD was 34% greater in males (p<0.05). At the 4% site Ix and SSI-p were 60% (p<0.05) and 117% (p=0.001) higher in males, respectively, while Ix at the 20% site was 84% higher in males (p=0.001). Although BMD or vBD measured either by DXA or pQCT showed a 6-29% trend of being greater in male specimens, differences only reached statistical significance at the 1/3-site with DXA and 4% cortical site with pQCT (25% and 29% higher in males, respectively, both p<0.05). Both trabecular structure indices (marrow fraction, Hmax and C.I.) and SOS were similar in male and female specimens. After mechanical testing, the distribution of fracture locations was found to be similar in the male and female specimens included in the final analysis. Failure load was 39% greater in males than in females (p=0.001). The mean Φ_{wrist} was 32% greater for female than male donors (p<0.01).

7.4.2 Predicting the Failure Load of the Distal Radius: Simple Regression Analysis

Bivariate regression analysis was performed for each bone variable and failure load of the distal radius (TABLE 7.1). The single predictors of failure load with the highest r^2-values were pQCT measures of SSI-p at the 4% and cortical bone content at the 4% and 20% distal radius sites (r^2=0.85, 0.85 and 0.82, respectively, all p<0.001) (FIGURES 7.4 and 7.5). Other pQCT measurements that predicted radial failure load very well were TOT-CNT at 4% and CRT-CSA at 20% (r^2=0.79 and 0.75, respectively, both p<0.001). DXA measurements of bone content at the ultradistal and one-third sites were also very strong predictors of distal radius failure load (r^2=0.76 and 0.71, respectively, both p<0.001). In general, bone mineral content measurements by either DXA or pQCT (4% and 20% sites) were more strongly correlated with failure load than their corresponding density measure (TABLE 7.1). SOS, DXR-BMD and phBMD were moderate to strong predictors of failure load (r^2=0.49, r^2=0.54, and r^2=0.63, respectively, all p<0.001). Geometric measurements of total, trabecular and cortical cross-sectional areas at the 4% site were only weakly correlated to radial failure load (r^2=0.24-0.30, p<0.05) and moment of inertia at 4% was not significantly correlated. In contrast, moment of inertia and cortical cross-sectional area at the 20% site were strongly correlated to failure load (r^2 = 0.58 and 0.75, respectively, both p<0.001). Trabecular structure indices at the 4% site, including porosity, Hmax and C.I., did not correlate with radial failure load.

CRT-CNT and SSI-p at the 4% site were significantly better predictors of radial failure load than all pQCT measures of the trabecular compartment, all cross-sectional

area measures, all trabecular structure indices and Ix and TOT-vBD at the 4% site, as well as CRT-vBD at the 20% site (p<0.05 for all). CRT-CNT and SSI-p were also significantly better predictors of failure load than SOS measured by ultrasound. Furthermore, SSI-p and CRT-CNT were significantly better predictors of failure load than DXR-BMD. To determine whether CRT-CNT at the 4% site is a significantly better predictor of failure load than AccuDEXA (using the correlation coefficients from in this study), a sample size of 32 would be needed. CRT-CSA at the 20% site was also found to be a significantly better predictor of failure load than CRT-CSA at the 4% site (p<0.05).

7.4.3 *Predicting the Failure Load of the Distal Radius: Multiple Regression Analysis*

Combinations of three bone variables, each measuring a different type of bone characteristic, were evaluated using stepwise regression analysis (TABLE 7.2). Measures of total bone mineral (density or content) by pQCT or DXA at the ultradistal region were moderate to strong predictors of failure load by themselves, with r^2-values ranging from 0.47 to 0.79. Measurements of geometry at the 20% site by pQCT added significantly to the predictive ability of total bone mineral variables alone to increase the R^2-value by 9-83%. Trabecular structure variables were then added to the combination of a bone mineral and bone geometry variable. Porosity and C.I. did not significantly add to any of the bone mineral and geometry combinations. Maximum hole size showed a trend to improve the R^2-value but only added significantly to DXR-BMD as the bone mineral density measure. With Ix or CRT-CSA as the 20% geometry variable, maximum hole size improved the

R^2-value by 18% (p<0.05). Coefficients of determination for these stepwise multiple regressions ranged from 0.71 to 0.86 (TABLE 7.2).

7.4.4 The Factor of Risk for Wrist Fractures

The mean Φ_{wrist} was 1.04 and 0.79 for female and male specimens, respectively. Thus, the women in this study had a greater probability of fracturing their distal radius upon falling from a standing height than the men, despite the increased height in the men. Moreover, there was a significant negative relationship between the UD radial T-score by DXA and Φ_{wrist} (r^2=0.73, FIGURE 7.6). Two of six individuals with a factor of risk greater than one had T-scores at the ultradistal radius that were less than –2.5, whereas the other four individuals with a factor of risk greater than one had T-scores that were below –1.5. Using an UD-BMD T-score criterion of less than -2.5, the sensitivity was 33% and specificity was 100% for predicting a Φ_{wrist} above 1. With an UD-BMD T-score criterion of less than -1.5, sensitivity and specificity were 100% and 93%, respectively.

7.5 Discussion

The primary goal of this study was to evaluate several peripheral bone assessment techniques to identify the bone variable or combination of bone variables that best predicts failure load at the distal radius. The single predictors with the highest correlation coefficients were pQCT measures of cortical bone mineral content at the 4% or 20% distal radius sites, and SSI-p at the 4% site, each explaining approximately 85% of the variance in failure load. We also found that measures of bone mineral content, by either

DXA or pQCT, showed a trend of being better predictors of failure load than their corresponding density measure. Our results suggesting that bone mineral content predicts failure load better than bone density is expected since our mechanical testing configuration was designed to test whole bone strength (i.e. structural properties), which is known to depend on bone size. Moreover, these results are consistent with previous investigations [Myers et al. 1991; Myers et al. 1993; Augat et al. 1998].

Multiple regression analysis showed that the prediction of failure load by measures of total bone density or content by DXA or pQCT could be improved upon by the addition of a bone geometry variable. This analysis also demonstrated that trabecular structure could improve upon the ability of the combination of DXR-BMD and a geometry variable to predict failure load.

The mean failure load of the 21 specimens (3231N ± 825) in this study was similar to those of earlier studies by Myers and Augat (2648-3390N) [Myers et al. 1991],[Augat, Reeb, and Claes 1996; Augat et al. 1998] and slightly higher than those of two previous studies reported by Myers and Spadaro (1780N and 1640N, respectively) [Myers et al. 1993; Spadaro et al. 1994]. In the Myers study, there was a much larger proportion of women (72%) than in the present study (45%)[Myers et al. 1993]. Spadaro et al. employed a quasistatic loading speed of 25mm/min, which is nearly 250 times slower than the displacement speed used in the current study and could alter the results since bone strength is dependent on strain rate[Carter and Hayes 1976; Spadaro et al. 1994].

These differences, along with variations in the loading configuration could help to explain the lower failure loads of these two studies relative to the failure load of our study.

In agreement with our results, two studies using pQCT also found that: 1) cortical measures of bone mineral content and CSA at proximal sites (10%-30% sites) appear to be better predictors of radial failure load than trabecular bone measures taken at the ultradistal 4% site, and 2) cortical CSA measured by pQCT at a proximal site seems to be a better predictor of distal radial failure load than cortical CSA measured at a more distal site, [Augat, Reeb, and Claes 1996; Augat et al. 1998]. This latter observation can be partially explained by the poor ability of pQCT to delineate the thin cortical shell at more distal radial sites, where the partial volume effect is large.

A recent study showed that QUS measurements of the phalanges were only moderate predictors of radial failure load (r^2=0.40-0.52), in agreement with the findings of this study for QUS of the distal radius (r^2=0.49) [Wu et al. 2000]. According to our results, however, the predictive ability of SOS measured by QUS of the distal radius is not as good as pQCT measures of CRT-CNT or SSI-p at 4% ($p<0.05$). Wu et al. (2000) reported pQCT measures of bone density, but did not report bone content or CSA measurements[Wu et al. 2000].

One of the strengths of this study is the inclusion of several different clinical assessment techniques measuring a number of different bone variables. Multiple regression was used to evaluate the ability of a combination of bone variables to predict radial failure load. The combinations that were evaluated included an average/total bone

mineral, a cortical bone geometry and a trabecular bone structure variable. Combining these variables incorporated both the amount of bone material present and also how that material was distributed in the cortical and trabecular compartments. Each of these factors has been shown to be an important contributor to failure load [Myers et al. 1991; Myers et al. 1993; Spadaro et al. 1994; Augat, Reeb, and Claes 1996; Augat et al. 1998; Gordon, Webber, and Nicholson 1998]. In our study, the addition of a bone geometry variable to a total bone mineral (content or density) variable increased the ability to predict failure load by 9-83%. R^2-values for these dual-variable stepwise regression tests varied from 0.71 to 0.86. Two-dimensional trabecular bone structure variables did not add significantly to either bone mineral measures or to a combination of a bone mineral and bone geometry variable, with the exception of maximum hole area which improved the R^2-value from 0.71 to 0.84. Therefore, even though trabecular structure indices measured by pQCT were generally poorly related to radial failure load in simple regression, some indices (specifically maximum hole size) may add information relating to bone strength which is independent of total bone density and cortical geometry. This finding supports previous work showing that maximum trabecular hole size and average hole size were able to add significantly to the ability of trabecular bone density (measured by pQCT) to predict failure load in excised cylindrical radial specimens [Gordon, Webber, and Nicholson 1998].

A considerable number of the cadaver arms available to us had to be excluded from analysis. Although age was found to be similar between the included and excluded radii,

excluded radii tended to have lower density. This could partly be explained by the fact that approximately one quarter of the excluded radii had previous fracture, likely associated with a lower bone density. In addition, lower density radii may be predisposed to fail in a manner that produces no fracture in the distal portion of the radius or creates indistinct failure points on the load-displacement curves of these specimens.

There are some limitations to our study. For example, although excising the radii allowed for more reproducible mechanical testing, the protocol is one more step removed from *in vivo* impact conditions during a fall on an outstretched hand. Normal load transfer to the radius is affected by the absence of muscle tone and soft tissue damping, which will in turn affect the forces applied to the radius during testing. Furthermore, this study employed a single loading configuration, which may not represent all falls. Our sample size was too small to determine if there was a significant difference between variables with very similar correlation values such as the best few predictors of failure load. A study with a much larger sample size would be needed to determine if there are any significant advantages of, for example, pQCT measures of SSI-p or CRT-CNT over DXA measures of UD-BMC in the prediction of radial failure load.

Since Colles' fractures generally occur at an earlier age than hip and spine fractures, and the presence of a Colles' fracture is associated with a greater risk for future fracture, then the radius may be a unique site for the early assessment of fracture risk. Based on the findings of our study, using SSI-p (4%), or CRT-CNT (4% or 20%) measures may improve identification of those individuals at highest risk for fracture.

Finally, we have presented a method for estimating a patient-specific factor of risk

for wrist fractures as the load applied to the wrist during a fall divided by the estimated

strength of that patient's radius. The potential advantage of this approach for prediction of

fracture risk is that the influence of both skeletal trauma and skeletal fragility are taken

into account, as each patient would have the adequacy of bone strength assessed with

respect to the estimated force imposed on the bone if that particular patient were to fall on

an outstretched hand. Albeit a small sample size, our data (FIGURE 7.6) suggests that the

T-score criteria for increased risk of fracture at the distal radius may be closer to -1.5 as

opposed to -2.5. Although theoretically appealing, the factor of risk for wrist fractures

should clearly be tested prospectively in a clinical cohort, and its ability to predict fracture

risk compared to that of BMD measurements alone.

In conclusion, the bone measurement techniques of QUS, DXR and phalangeal bone

density are moderate to good predictors of distal radial failure load (r^2=0.49-0.63). DXA

measurements were also good predictors of failure load with UD-BMC appearing to be

the best, explaining 76% of the variance in failure load. The best single predictors,

however, seem to be pQCT measures of cortical bone content or geometry, specifically,

CRT-CNT at 4% or 20% site, and SSI-p at the 4% site which each explain approximately

85% of the variance in radial failure load. Measures of radial geometry at the 20% site by

pQCT add significantly to the ability of total bone mineral measures by DXA or pQCT to

predict failure load. However, this combination of two variables still can not outperform

SSI-p (4%) and CRT-CNT (4% or 20%) of the forearm by pQCT as the best single predictors of failure load at the distal radius.

TABLE 7.1: Descriptive statistics (mean ± SD) for 21 specimens by gender and coefficient of determination (r^2-value) for bivariate regression of bone variables for the total sample (n=21) with failure load.

Bone Measurement Technique	Variable Measured	Female (n=9)	Male (n=12)	r^2-value
	Age (y)	76.6 ± 15.4	75.2 ±11.9	NS
	Weight (kg)	71.9 ± 18.6	66.6 ± 15.4	NS
	Height (m)	1.64 ± 0.09 (n=8)	1.73 ± 0.09	NS
	Failure Load (N)	2642 ± 397	3673 ± 792	N/A
	Factor of Risk	1.045 ± 0.170 (n=8)	0.793 ± 0.209	N/A
DXA of forearm	UD-BMC (g)	1.28 ± 0.40	1.76 ± 0.38	0.76 [a]
	UD-BMD (g/cm^2)	0.367 ± 0.103	0.442 ± 0.078	0.60 [a]
	UD T-score	-1.32 ± 1.78	-0.01 ± 1.34	0.60 [a]
	1/3-BMC (g)	1.63 ± 0.26	2.33 ± 0.51	0.71 [a]
	1/3-BMD (g/cm^2)	0.595 ± 0.097	0.741 ± 0.149	0.58 [a]
	1/3 T-score	-1.65 ± 1.61	0.79 ± 2.48	0.58 [a]
pQCT - 4% site	TOT-CNT (mg)	87.7 ± 22.1	136.2 ± 29.3	0.79 [a]
	TOT-vBD (mg/cm^3)	344 ± 96	397 ± 64	0.47 [b]
	TRAB-CNT (mg)	20.8 ± 7.0	36.1 ± 13.4	0.53 [a]
	TRAB-vBD (mg/cm^3)	182 ± 68	194 ± 48	0.22 [c]
	CRT-CNT (mg)	60.5 ± 13.7	89.7 ± 16.8	0.85 [a]
	CRT-vBD (mg/cm^3)	542 ± 138	700 ± 114	0.69 [a]
	TOT-CSA (mm^2)	257.1 ± 24.8	345.7 ± 73.3	0.30 [c]
	TRAB-CSA (mm^2)	117.4 ± 22.6	184.9 ± 55.8	0.30 [c]
	CRT-CSA (mm^2)	113.0 ± 11.7	127.9 ± 13.1	0.24 [c]
	Ix (mm^4)	2672 ± 608	4288 ± 1780	NS
	SSI-p (mm^3)	205 ± 105	444 ± 144	0.85 [a]
pQCT - 20% site	CRT-CNT (mg)	69.7 ± 13.2	99.5 ± 21.6	0.82 [a]
	CRT-vBD (mg/cm^3)	979 ± 101	1044 ± 109	0.53 [a]
	CRT-CSA (mm^2)	70.9 ± 8.1	94.5 ± 14.3	0.75 [a]
	Ix (mm^4)	798 ± 210	1471 ± 487	0.58 [a]
AccuDEXA	phBMD (g/cm^2)	0.321 ± 0.078	0.428 ±0.080	0.63 [a]
QUS	SOS (m/s)	3995 ± 122	4088± 178	0.49 [a]
DXR	DXR-BMD (g/cm^2)	0.475 ± 0.063 (n=6)	0.553 ± 0.095 (n=11)	0.54 [a]
pQCT Structure (4% site)	Porosity	0.506 ± 0.017	0.502 ± 0.019	NS
	Connectivity Index	9.25 ± 3.67	8.69 ± 2.59	NS
	Hmax (cm^2)	0.736 ± 0.106	0.843 ± 0.223	NS

Significance for regression: [a] = p< 0.001, [b] = p<0.01, [c] = p<0.05, NS = not significant

TABLE 7.2: Bivariate or stepwise regression with one, two or three different bone variables and the corresponding coefficients of determination.

Bone Mineral Alone	r^2-Value[a]	Bone Mineral Mineral + Bone Geometry[c] ± Trabecular Structure[d]	R^2-Value[b]
UD-BMD	0.60	UD-BMD + CRT-CSA	0.86
UD-BMC	0.76	UD-BMC + CRT-CSA	0.85
4%TOT-vBD	0.47	4%TOT-vBD+ CRT-CSA	0.86
4%TOT-CNT	0.79	4%TOT-CNT+ CRT-CSA	NS
phBMD	0.63	phBMD + Ix	0.75
DXR-BMD	0.54	DXR-BMD + Ix	0.71
		DXR-BMD + Ix + Hmax	0.84

[a]All coefficients of determination found significant at $p < 0.05$
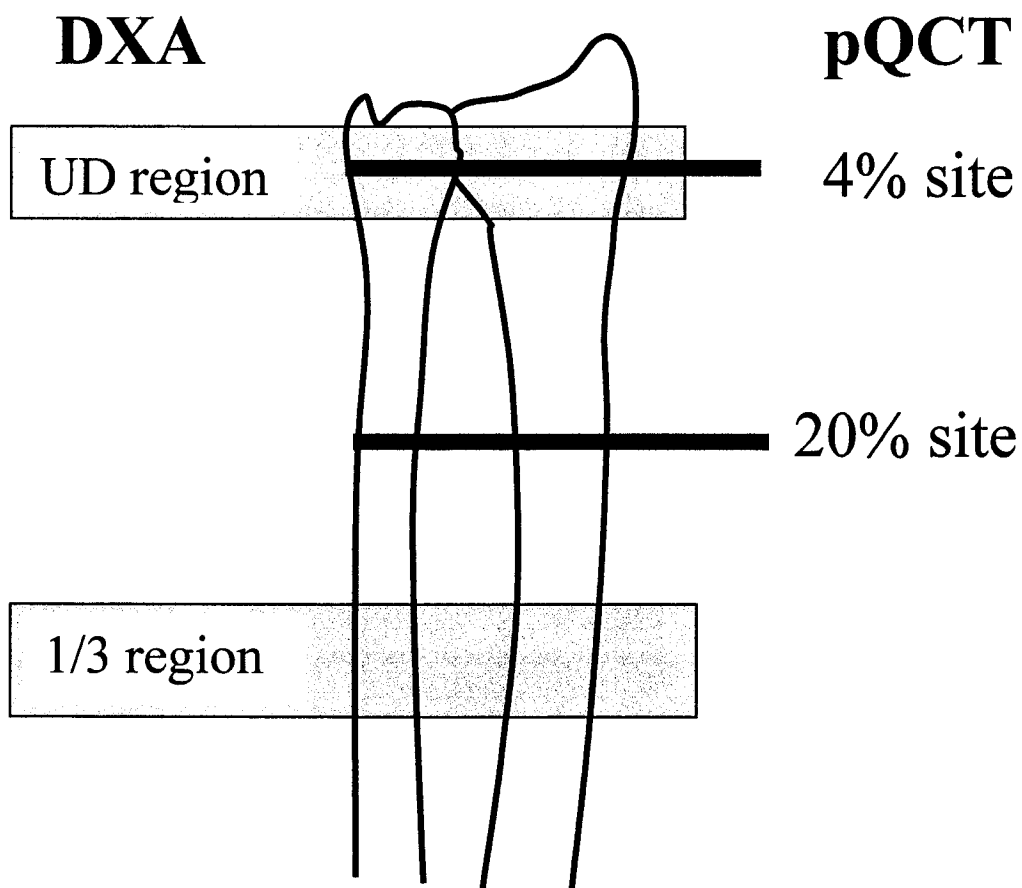
[b]Addition to r^2-value considered significant at $p < 0.05$, NS = not significant

[c]All geometry measurements are taken from the 20% site by pQCT

[d]Trabecular structure variables significantly added to R^2-value for DXR-BMD combinations only

FIGURE 7.1

DIAGRAM SHOWING THE MEASUREMENT LOCATIONS FOR
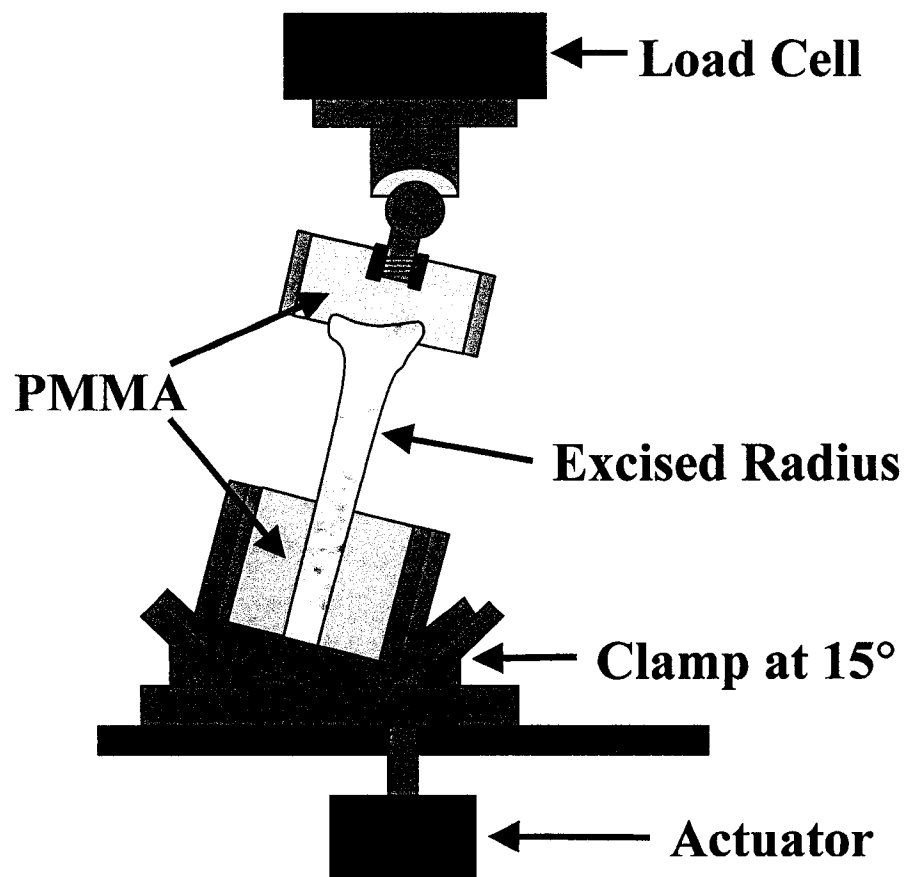DXA AND PQCT SCANS

UD = ULTRADISTAL, 1/3 = ONE-THIRD DISTAL.

FIGURE 7.2

DIAGRAM OF THE MECHANICAL TESTING CONFIGURATION
TO DETERMINE RADIAL FAILURE LOAD

PMMA = POLYMETHYLMETHACRYLATE

FIGURE 7.3

GRAPHS OF REPRESENTATIVE LOAD-DISPLACEMENT CURVES
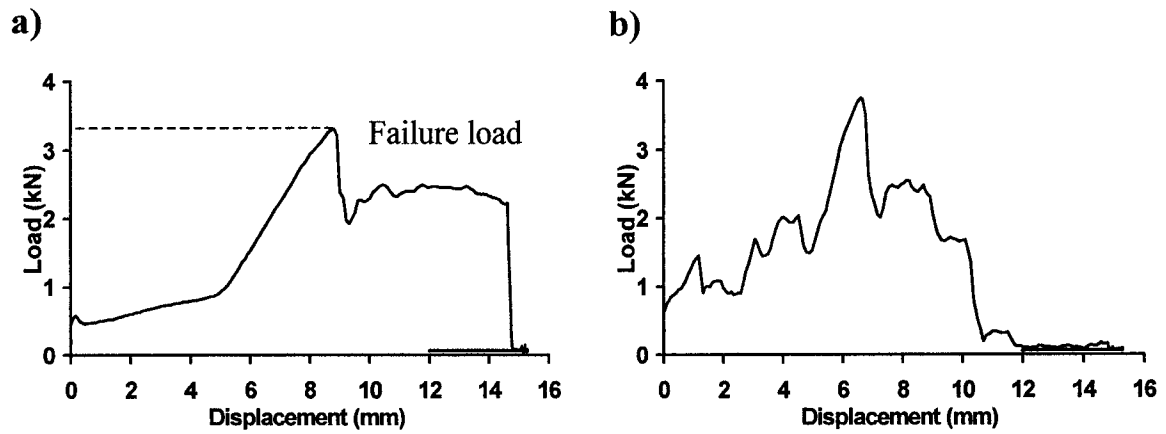
a) A Standard Load-Displacement Curve With a Distinct Failure Point
b) A Load-Displacement Curve Showing an Indistinct Failure Point

FIGURE 7.4

GRAPH OF CORTICAL CONTENT BY PQCT AT THE 4% SITE VERSUS
RADIAL FAILURE LOAD (Regression line and r^2-value shown for total sample)

CRT-CNT vs Radial Failure Load

FIGURE 7.5

GRAPH OF SSI-P BY PQCT AT THE 4% SITE VERSUS RADIAL FAILURE LOAD
(Regression line and r^2-value shown for total sample)

SSI-p vs Radial Failure Load
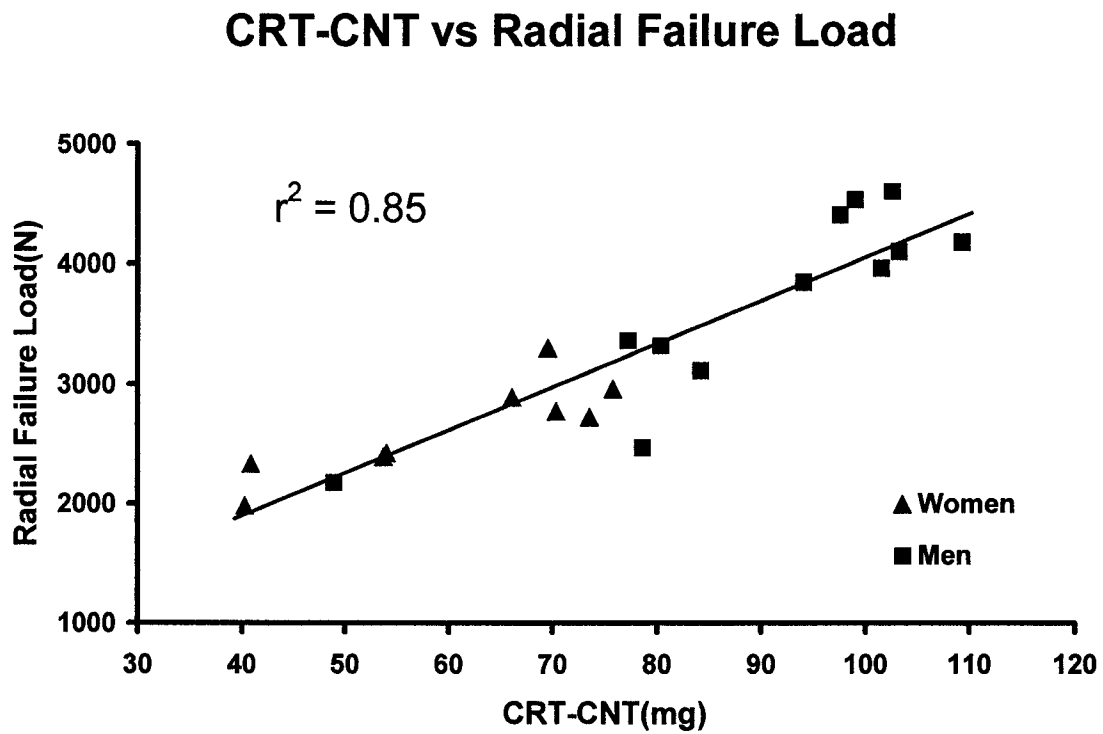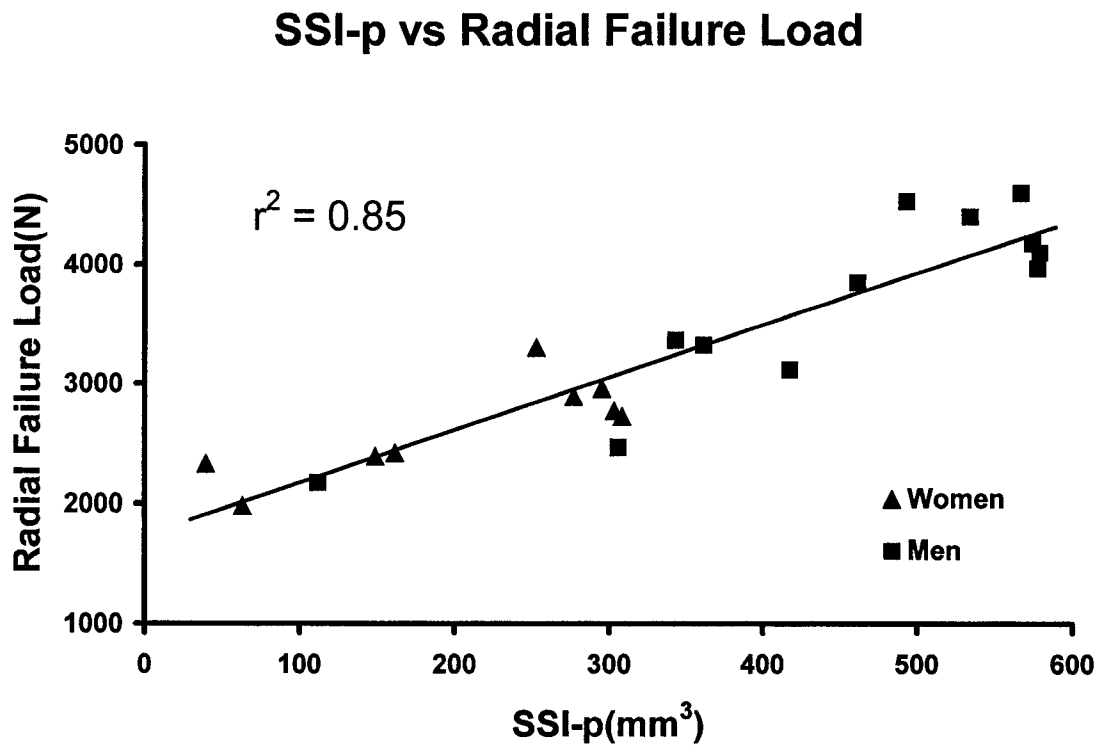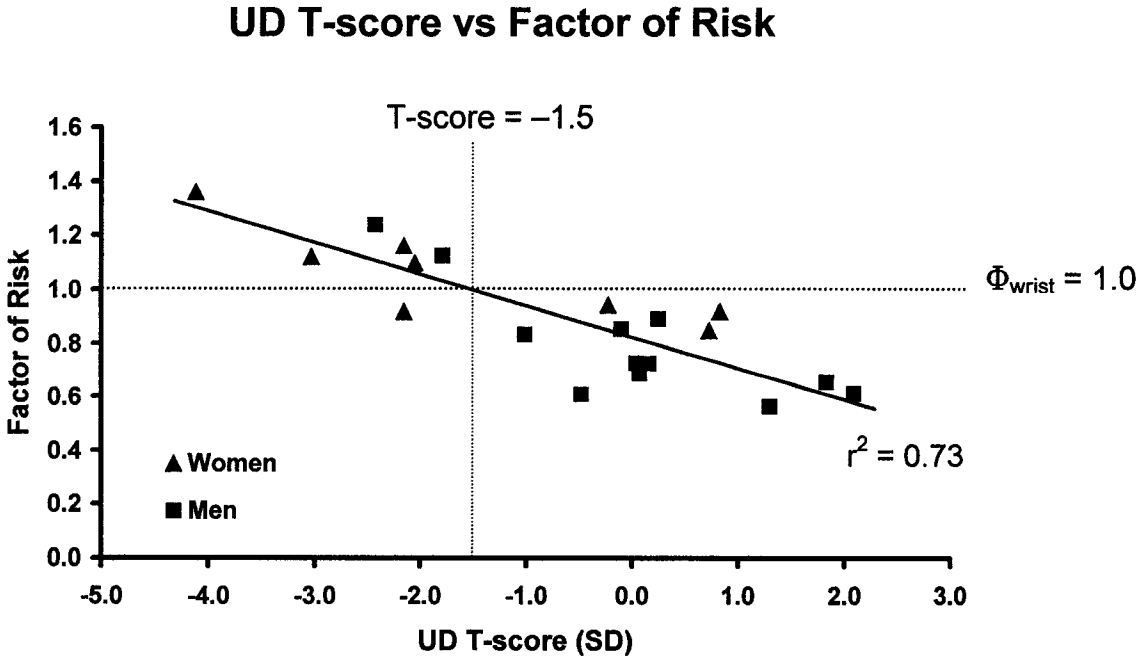
FIGURE 7.6

GRAPH OF ULTRADISTAL T-SCORE BY DXA VERSUS FACTOR OF RISK
FOR THE WRIST (Regression line and r^2-value shown for total sample)

UD T-score vs Factor of Risk

7.6 Appendix

TABLE OF TERMS FOR BONE MEASURES

Term	Meaning	Measurement Technique	Units
UD-BMC	bone mineral content of ultradistal region of radius	DXA	g
UD-BMD	bone mineral density of ultradistal region of radius	DXA	g/cm^2
1/3-BMC	bone mineral content of 1/3-distal region of radius	DXA	g
1/3-BMD	bone mineral density of 1/3-distal region of radius	DXA	g/cm^2
TOT-CNT	bone content of total 2.5mm slice of radius (total = cortical plus trabecular regions)	pQCT	mg
TOT-vBD	volumetric bone density of the total 2.5mm slice of radius	pQCT	mg/cm^3
TRAB-CNT	bone content of the trabecular region of the 2.5mm slice of radius	pQCT	mg
TRAB-vBD	volumetric bone density of the trabecular region of the 2.5mm slice of radius	pQCT	mg/cm^3
CRT-CNT	bone content of the cortical region of the 2.5mm slice of radius	pQCT	mg
CRT-vBD	volumetric bone density of the cortical region of the 2.5mm slice of radius	pQCT	mg/cm^3
TOT-CSA	cross-sectional area of the total radial bone slice	pQCT	mm^2
TRAB-CSA	cross-sectional area of the trabecular bone in the radial bone slice	pQCT	mm^2
CRT-CSA	cross-sectional area of the cortical bone in the radial bone slice	pQCT	mm^2
Ix	moment of inertia of the radial cortex around x-axis [$= \sum$ in-plane voxel area * (distance from x-axis)^2]	pQCT	mm^4
SSI-p	polar stress-strain index of the radial cortex (z-axis) [=polar moment of inertia/max distance from centre * cortical density/physiologic bone density]	pQCT	mm^3
phBMD	mean bone mineral density of the middle phalanges of the of the 2nd and 4th digits	AccuDXA	g/cm^2
SOS	speed of sound along the radius	Ultrasound	m/s
DXR-BMD	bone density calculated from cortical thickness of ulna, radius and middle 3 metacarpals from x-ray	Digital x-ray radiogrammetry	g/cm^2
Porosity	fraction of trabecular bone that is marrow [marrow area/(marrow area + bone area)]	Structure analysis of pQCT image	unitless
Connectivity Index	(#of nodes - #of free ends - #of isolated points)*100 / length of the trabecular network	Structure analysis of pQCT image	unitless
Hmax	area of the largest trabecular marrow pore	Structure analysis of pQCT image	cm^2

CHAPTER 8: INTEGRATED D ISCUSSION AND CONCLUSIONS

8.1 Bringing it All Together

There are 2 main studies included in this thesis, the prospective clinical trial following the bone effects of medications for osteoporosis and the *in vitro* radial cadaver study evaluating the ability of different bone variables to predict radial bone strength. The baseline data of the prospective trial could also be considered a separate sub-study. The synthesis that is of principle interest, is the combination of the *in vivo* prospective clinical trial data with the *in vitro* radial bone strength data. This combination will be explored first, followed by other patterns or points of interest that emerge when considering the results from the baseline, prospective and *in vitro* studies together.

The results from the prospective *in vivo* clinical pharmacological study and the results from the *in vitro* bone strength study have been discussed separately in their respective chapters, six and seven. When considered together, new information may be extracted from their results. Specifically, a linkage between which bone variables predict radial bone strength the best and which drug treatment regimens improve these identified radial bone variables the most. This permits an indirect assessment of how each pharmacological regimen affects bone strength at the distal radius. Cortical bone content at the 4% site of the distal radius was the single predictor of radial bone strength on

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mechanical testing that had the highest prediction value (r^2=0.85) and was also measured in the prospective clinical trial. In the clinical trial, A+H, E+H and HRT groups were all found to have gained significantly more CRT-CNT than the control group over the 2-year study. On the other hand, etidronate alone or alendronate alone, did not show significant gains in CRT-CNT over the two year trial versus the control group. Considering the data from these two studies together suggests that over the two-year trial, the two combination therapies of A+H and E+H, as well as HRT alone, increased bone strength at the distal radius in the women in these treatment groups more than the women in the control group.

In the control group, significant losses were only found in CRT-CNT (-2.3%, p=0.029) and TOT-CNT (-2.0%, p=0.005) at the 4% distal radial site after two years had elapsed. Taken together with the mechanical loading study (total content was the pQCT variable at the 4% site that had the second largest correlation value with radial failure load, r^2=0.79) these results imply that women in the control group lost bone strength at the distal radius over the two-year trial.

By examining the performance of the different bone variables in all of the studies in this thesis together, certain patterns emerge. For example, it appears that total and cortical bone content measurements generally performed better than the equivalent bone density measures at the distal radius in many respects. The trends in the results suggesting this are:

1) TOT-CNT and CRT-CNT had higher Z-statistics than TOT-vBD and CRT-vBD for discriminating between osteoporotic and osteopenic groups in the baseline analyses of the clinical trial

2) TOT-CNT and CRT-CNT had higher r^2-values than their respective bone density measures when correlated with radial failure load in the *in vitro* mechanical loading study

3) TOT-CNT and CRT-CNT showed significant losses from baseline in the control group over the two-year clinical trial whereas total and cortical bone density variables did not significantly change over the length of the prospective trial

One instance where this pattern does not hold is in the women of the treatment groups in the prospective study. When considering the women on anti-resorptive therapies in the prospective clinical trial, TOT-vBD and CRT-vBD showed significant changes over baseline that were greater than those seen in TOT-CNT and CRT-CNT.

When examining the different pQCT measures from a clinical perspective the question becomes, which pQCT bone measure or measures should be utilized at the distal radius? CRT-CNT and TOT-CNT had the highest z-statistics of all non-DXA measurements in the clinical baseline report, suggesting their ability to discriminate between osteoporotic and osteopenic groups was better than that of any other pQCT or anthropometric measure. These findings, taken together with the results from the control group in the prospective clinical trial and the *in vitro* bone strength study, indicate that

pQCT measurements of bone mass or content in the total and cortical compartments of

the distal radius may be the most sensitive of the pQCT measures for:

1) classification (diagnosis)

    •   suggested by the baseline sub-study

2) change over time (sensitivity to change or responsiveness)

    •   suggested by the control group in the prospective clinical trial

3) prediction of bone strength (prognosis)

    •   suggested by the *in vitro* mechanical testing study

In contrast, measures of the trabecular compartment appear to be more sensitive to

changes in the distal radius that occur when anti-resorptive treatment is taken. The

trabecular compartment showed the largest changes from baseline, especially TRAB-

CNT (changes up to -5.0% from baseline). Furthermore, when the total and cortical

compartments are considered, bone density measures seem to be more sensitive to change

with anti-resorptive treatment than their corresponding content measures. The data from

the pQCT measurements at the distal radius do suggest that endocortical resorption is a

mechanism of bone loss in the control group, whereas endocortical apposition is a

mechanism for bone gain in the treated group (see section 6.5.2). However, the

differences between changes in the control group and treatment group in the various bone

compartments also suggest that there are different mechanisms for bone mass changes

and reorganization of bone mineral at the distal radius when bone is under the influence

of the aging process (control group) versus the influence of anti-resorptive medications

(treatment group). One process is not simply the reversal of the other. In other words, although anti-resorptive treatments have been associated with gains in bone mass after it has been lost, these therapies do not necessarily act by directly reversing the aging process in the skeleton. All of this information suggests that different measures may be appropriate for different circumstances depending on the purpose of the bone measurement (diagnosis, prognosis or evaluation) and the patient group to be tested. It is, therefore, plausible that different measures may be appropriate for following bone changes with age than bone changes with anti-resorptive therapies.

It should be noted that future studies with larger sample sizes are needed to determine if any of these pQCT bone variables are statistically significantly better than the other pQCT variables for each measurement purpose.

8.2   Conclusions

8.2.1   *Baseline Data from the Prospective Clinical Trial*

➢ As a group, the women considered to be osteoporotic are older, lighter and have a distal radius with less bone mass, a thinner cortical shell, and a trabecular network that has larger holes and is more poorly connected than the women in the osteopenic group.

➢ *In vivo* trabecular structure measures by pQCT at the distal radius are able to significantly discriminate between osteopenic and osteoporotic groups of postmenopausal women. These structure indices (CI, Ha, Hm) are better

discriminators than height and total and trabecular cross-sectional area at the distal radius.

➢ The following trends are also found at the distal radius:

◆ PQCT measures of the cortical compartment are better than the equivalent measures of the trabecular compartment at discriminating between osteoporotic and osteopenic groups

◆ PQCT measures of bone content are generally better than their corresponding density measures at discriminating between osteoporotic and osteopenic groups

◆ The pQCT measure of total bone content may be the best of all pQCT measures at discriminating between osteoporotic and osteopenic groups

◆ Age and weight are better than height at discriminating between osteoporotic and osteopenic groups

8.2.2 Longitudinal Data from the Prospective Clinical Trial

➢ Women taking any anti-resorptive therapy significantly gained bone density as measured by DXA at the lumbar spine and femoral neck at one and two years of treatment. These women also significantly gained cortical bone density at the distal radius at one and two years and total bone density at one year with a trend for a gain at 2 years (p=0.074).

➢ At the end of the 2-year trial, subjects taking a combination therapy (HRT with either alendronate or etidronate) had greater increases in femoral neck and lumbar spine BMD than subjects who took only one anti-resorptive therapy. The distal radius also

showed the similar trend of combination therapy producing greater gains in BMD
than a single anti-resorptive therapy, but was only statistically significant for total
bone content over the 2-year trial.

> HRT, etidronate and alendronate therapies, alone or in combination, were found to
produce greater percentage increases in bone density at the lumbar spine than at the
femoral neck or distal radius.

> A+H group had the lowest baseline hip T-score, spine T-score, radial volumetric bone
density and content and worst indices of radial bone structure, but had the greatest
increases in hip and spine BMD.

8.2.3 In Vitro *Mechanical Loading Study*

> The pQCT measure of CRT-CNT is a significantly better predictor of radial failure
load than either speed of sound measured by ultrasound or bone density measured by
digital x-ray radiogrammetry.

8.3 Application to the Clinic

The first step for a clinician is to determine which bone assessment technique to use
as a surrogate measure for fracture risk in the care of their patient's skeletal health. This
thesis and other studies have shown that anti-resorptive therapies generally cause smaller
gains in BMD at the distal radius than at the spine or hip as measured by areal
density[Lufkin et al. 1992; Liberman et al. 1995; Black et al. 1996; Recker et al. 1999;
Delmas et al. 2000; Harris et al. 2001]. It has also been found that changes in forearm

BMD by DXA do not predict changes in either hip or spine BMD with

alendronate[Bouxsein, Parker, and Greenspan 1999]. For this reason, it has been

suggested that forearm BMD can not be used to follow treatment response[Bouxsein,

Parker, and Greenspan 1999]. Results from the present study of pQCT measured TOT-

vBD and TOT-CNT showing no significant change over baseline at the two year time

point (although significant changes of 1.5% and -1.1%, respectively were seen at 18

months) also support this view. In contrast, significant changes found in the trabecular

and cortical compartments (1.7% to 4.5%) in this thesis contest that the forearm may

indeed be a valuable site for monitoring response to therapy. These changes in the

trabecular and cortical compartments with anti-resorptive therapies must, however, be

measured by pQCT since they can not be detected using DXA. PQCT scanners are also

smaller and less expensive than DXA machines and thus provide a viable option to the

clinician who wishes to directly monitor treatment effects in his or her office. Results

from the studies in this thesis also showed that pQCT measures of TOT-CNT and CRT-

CNT had the highest correlation with failure load in the mechanical loading study,

discriminated between groups of osteopenic and osteoporotic women and measured

significant losses from baseline in women who were not taking osteoporotic medication.

Furthermore, measurements at the forearm have been shown to be better predictors of

future wrist fracture than measures at the spine or hip by DXA and it may be

hypothesized that this would hold true for pQCT at the forearm[Marshall, Johnell, and

Wedel 1996]. If however, the clinician is interested in monitoring response to treatment at

the hip and spine, or is interested in fracture prediction at these sites, then measurements should still be made at the hip and spine. DXA at the hip and spine have been shown to be the best predictors of fracture at the hip and spine, respectively. Response to therapy at the radius measured by pQCT has not been shown to correlate with treatment response at either the hip or spine. The application to the clinic is that, although pQCT measurements at the distal radius may be useful for many purposes at the forearm, measurements at the spine and hip are still required to address site-specific questions at central locations until more research is done.

After choosing a method of assessment, the clinician must decide which medication to prescribe to reduce fracture risk for their patients. Many factors should be considered such as the patient's medical history and contraindications. Results from this thesis, however, focus on the bone effects of different anti-resorptive medications alone or in combination. Studies have shown that a small increase in BMD gives rise to a large reduction in fracture risk as outlined in section 3.3. Research has shown that there is a gradient effect where larger increases in bone mass lead to larger reductions in fracture risk[Hochberg et al. 1999; Wasnich and Miller 2000]. For example, a 4% gain in spine BMD gave a 38% reduction in vertebral fracture rate, whereas an 8% gain in spinal BMD gave a 54% reduction in vertebral fracture incidence[Wasnich and Miller 2000]. However, as the amount of BMD gained increases, the magnitude of further fracture reduction decreases. For example, there is a 38% reduction in fracture risk for the initial 4% gain in BMD, whereas with the subsequent 4% gain in BMD (i.e. from 4-8%) there is

only a 16% further reduction in fracture risk[Wasnich and Miller 2000]. Application of this information to the patient helps to direct clinical decision-making. With all other factors being equal between different drugs, the data suggest choosing the single drug that increases bone density the most, hopefully also lowering fracture risk the most. If however, there are marked differences between medications in other factors such as cost, side effects or contraindications, then the physician and patient must weigh these with the added benefits of choosing a drug that may produce larger BMD gains and a greater reduction in fracture risk.

When considering combination therapy versus single therapy, there is a deficit of research focussing on fracture reduction. There is no direct information of whether combination therapy reduces fracture rate more than single therapy, nor is there data showing a reduction in fracture rate with combination therapy versus placebo. Without this information it can only be hypothesized that the greater gain in BMD with combination therapy over single therapy would lead to larger reductions in fracture risk. This hypothesis needs to be addressed in a large randomized controlled trial that is powered to evaluate fracture risk. Keeping this limitation in mind while applying the results of this thesis that suggest a potential benefit of combination therapy, the following recommendation is given: consideration of combination therapy should perhaps be limited to patients who are either at great risk of fracture (i.e. very low BMD, previous fracture, high propensity to fall, etc.) or who have shown an insufficient response to single therapies.

8.4 Limitations

8.4.1 Prospective Clinical Trial:

Effects of Pharmacological Treatments for Osteoporosis on the Distal Radius

The first limitation of the clinical trial is that the subjects were not randomized to the treatment regimens and the control group, and that neither the rater nor the subjects were blinded. Secondly, the sample size was adequate to detect changes from baseline and differences from placebo when all anti-resorptive therapies were combined into one group, but this group was heterogeneous, with different medications having different mechanisms of action. When the subjects were divided into the 6 individual treatment groups the sample sizes were too small, especially in the combination therapy groups, to detect with pQCT, significant differences between the individual treatment regimens. Other limitations are that there was no statistical adjustment for baseline BMD or for the HRT group being younger and the 2-Tx group having a lower baseline BMD. As well, there were multiple statistical comparisons in the analysis (27) that could lead to 1-2 significant findings by chance alone at the $\alpha=0.05$ level. Also, the subjects were not all measured with the same DXA scanner and their diet and exercise were neither controlled nor adjusted for in the analysis.

8.4.2 In Vitro *Mechanical Loading Study*

An important limitation in this study was the small sample size of twenty-one cadaver forearm specimens that were included in the analysis. Further limitations include a single loading configuration in the mechanical testing and that the study was *in vitro*, without the *in vivo* effects of soft tissue dampening and muscle reaction forces.

8.5 Future Work

The method used in this thesis to separate trabecular bone from cortical bone in the pQCT distal radial scan (iterative contour detection) accounts for all of the mineral in the radial bone slice and therefore allows for identification of shifts in bone mass from one compartment to another. Since the border between cortical and trabecular bone changes with the effects of time/aging or with treatment, then the bone that is considered to be in each compartment also changes. Another method of analyzing the pQCT radial bone scan, which was not used in this thesis, selects the inner 45% of the cross-section to be the trabecular bone compartment. If the data from the prospective trial were reanalyzed using this latter approach, then this analysis would give a better indication of what is really happening to the inner trabecular network and likely be able to determine if there is trabecular thinning or thickening occurring. Perhaps both analyses are required routinely since they offer different information. The iterative contour detection identifies mass shifts from one compartment to another and changes in the endocortical surface, while the inner 45% trabecular core provides information regarding the trabecular bone that is not

affected by endocortical changes and can therefore likely detect architectural changes such as an increase or decrease in trabecular plate thickness or hole size.

This thesis has used *in vivo* measurements to determine shifts in bone mass between cortical and trabecular compartments, and to assess changes in trabecular structure due to aging or anti-resorptive treatment. An interesting next step would be to evaluate the effects of bone formation medications such as parathyroid hormone or fluoride on bone mass shifts and trabecular structure and how these effects differ from those of the anti-resorptive medications.

Other work following this thesis could include utilization of different measurement techniques such as the next generation of pQCT scanners or MRI. These more modern pQCT machines have the ability to perform serial-stacked measurements and also scan at a faster rate. This would allow for a more representative portion of the distal radius to be followed and likely would improve reproducibility due to the larger volume and reduced scanning time. Peripheral MRI would also be another avenue to pursue since true volumetric measurements can be made without ionizing radiation. Trabecular structure information in the coronal plane may give additional information to help determine what is occurring at the distal radius with aging or treatment.

This thesis has shown bone mass shifts to and from cortical and trabecular compartments, but only at the wrist. It would be worthwhile to see if these same changes also occur at other sites such as the hip and spine. Like the distal radius, the proximal femur and vertebrae are also important sites of osteoporotic fracture, but these two sites

are different from the wrist in that they are weight-bearing sites. Whole body CT scanning could be used to detect bone mass shifts and structural changes, but these machines have many limitations such as expense, high clinical demand and larger radiation doses than their peripheral cousin. Another option would be MRI, which would yield qualitative information such as the trabecular structure but not quantitative information such as amount of bone mineral.

With regards to the prediction of radial bone strength, future work should include a prospective *in vivo* study evaluating the ability of the pQCT radial bone measurements to predict wrist fractures. The *in vitro* mechanical loading study in this thesis evaluated the ability of bone measurements to predict radial failure load in a single loading configuration. The more clinically relevant question is whether and how well, these bone variables predict actual fractures occurring naturally in everyday life. This would require a large prospective trial focussing on the measurement variables that best predict radial fracture in a specified population, such as postmenopausal women, that has a relatively high incidence of low-trauma radial fractures. Although many studies have investigated the ability of forearm bone mineral density measurements to predict fractures, the majority of these studies used DXA or SPA[Marshall, Johnell, and Wedel 1996; Duppe et al. 1997]. Results from the *in vitro* mechanical loading study of this thesis, and the work of others suggests that pQCT radial bone variables may be better predictors of bone strength than SPA or DXA variables and thus should be evaluated for prediction of radial

fractures prospectively[Myers et al. 1991; Myers et al. 1993; Augat, Reeb, and Claes 1996; Augat et al. 1998].

8.6 Summary

Anti-resorptive therapies alone, or in combination, significantly increase bone density at the lumbar spine, femoral neck and distal radius. Combination therapy has been shown to increase bone mass at the hip, spine and radius more than a single anti-resorptive treatment. The combination of alendronate plus HRT produced significantly greater gains in bone density than either etidronate or HRT alone at the hip and spine and greater than alendronate alone at the hip after two years. Women who were not on any treatment significantly lost bone mass at the distal radius and showed a trend for bone loss at the spine (p=0.062). Overall, anti-resorptive therapies were found to significantly increase bone mass or density versus no treatment at the hip, spine and distal forearm. This thesis provides information supporting the use of pQCT measurements at the distal radius to follow bone changes with age and to evaluate the effects of anti-resorptive treatments at the forearm.

The ability to detect shifts in bone mass between trabecular and cortical compartments of the distal radius has facilitated the development of theoretical mechanisms for *in vivo* bone changes at the distal radius with both age and anti-resorptive therapies. The proposed mechanisms for bone changes at the distal radius with age are endosteal resorption and trabecular thinning. With anti-resorptive treatment, the proposed mechanisms are decreased intracortical porosity, endosteal apposition and perhaps

trabecular thinning and/or periosteal resorption that lead to the changes measured by pQCT at the distal radius.

PQCT measures at the distal radius can also be used to predict *in vitro* bone strength. These measurements can therefore aid in the estimation of fracture risk and may help to assess how this risk changes with either age or drug therapy.

Letter of Permission

The following letter was faxed on May 18th, 2003 to Springer-Verlag London Ltd requesting permission to include in this thesis the article "Predicting failure load of the distal radius", which has been accepted for publication in Osteoporosis International. A follow-up email was sent on June 13th, 2003 with the letter attached. As of June 25th, 2003 there has been no response.

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