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The Effect of Varying Bisphosphonate Treatment on Changes in Bone Microdamage in Osteoporotic Women

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THE EFFECT OF VARYING BISPHOSPHONATE TREATMENT ON CHANGES IN BONE MICRODAMAGE IN OSTEOPOROTIC WOMEN

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in Biomedical Engineering in the College of Engineering
at the University of Kentucky

By

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Lexington, KY

Director: Dr. David Pienkowski

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2016

ABSTRACT OF THESIS

THE EFFECT OF VARYING BISPHOSPHONATE TREATMENT ON CHANGES IN BONE MICRODAMAGE IN OSTEOPOROTIC WOMEN

Bisphosphonates (BPs) are used for the treatment of osteoporosis. This study evaluated changes in bone microdamage with BP treatment duration. Fifty-one iliac crest biopsies were obtained from Caucasian women, ages 41 to 87 years, who were previously diagnosed and treated for osteoporosis with oral BPs for 1-16 years duration. Patients diagnosed with any disease, drug, or substance abuse that may affect bone metabolism were excluded.

Bone samples were sectioned, stained, and histologically examined using light and fluorescence microscopy. Bone area, number and length of microcracks were quantified. Following adjustment for age, BMD, BV/TV, trabecular thickness, and turnover, regression analysis revealed a relationship between microcrack density and treatment duration ($p=0.018$). No significant relationship was observed between microcrack length and treatment duration.

This study provides novel data relating microdamage with varying BP treatment duration in human bone. Given information from other studies showing that microdamage amounts are related to changes in bone biomechanics, the BP treatment duration related changes in microdamage shown offer new information that may help optimize osteoporosis treatment.

KEYWORDS: microdamage, bisphosphonate treatment, microcrack density

Stefanie Lynn Pagano

May 2, 2016

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Chapter I Introduction and Background

1.1 Introduction

As healthcare technology advances, therapies are being developed that increase the human life span. With an increase in life expectancy, there is a simultaneous need for continued quality of life. By the year 2030, the number of Americans that live past 65 years of age will have almost doubled [1, 2] and underlying this profound change in demographics is an increased need to address and treat the medical concerns that occur in this aging population. One of the most noteworthy of these medical concerns is osteoporosis, a systemic loss of bone from the skeleton. The prevalence of osteoporosis has continued to rise over time and will only continue to do so as the elder population expands (Fig. 1.1) [2].

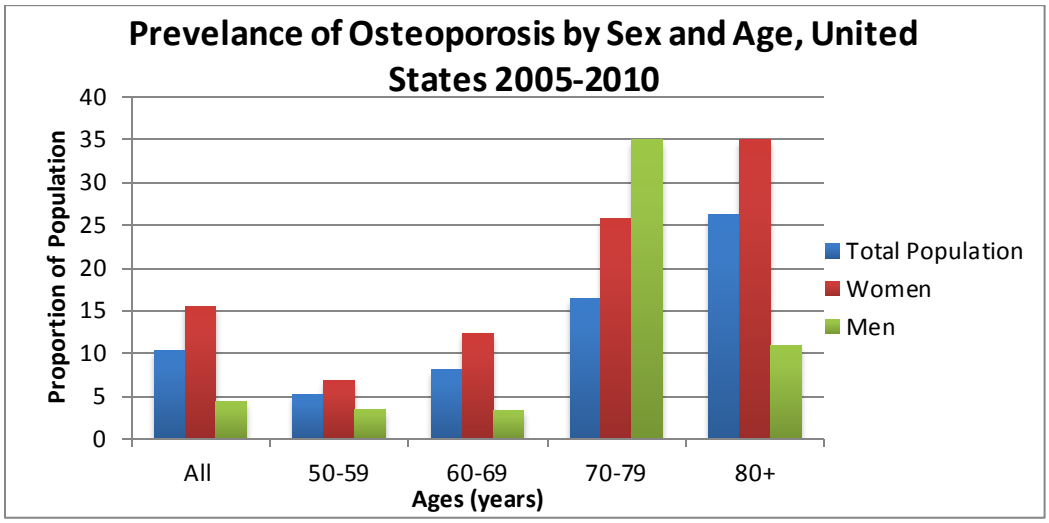

Figure 1.1. Prevalence of Osteoporosis. Reproduced with permission from Wright NC, Looker et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. JBMR 2014, 2520-2526. Copyright John Wiley & Sons, Inc.

More specifically, osteoporosis is a worldwide health problem with more than 200 million people affected [5]. In the United States, more than 10 million people have been diagnosed with osteoporosis, and more than 18 million are at risk to develop the disease [5]. Based off data gathered from 1990-2011, the hospitalization costs for a hip fracture ranged from $8,358 to $32,195 [6]. In the year 2002, the estimated mean cost of treating an osteoporotic-related bone fracture in the United States was $8,600 [6]. Additionally in

that year, more than 1.6 million people experienced an osteoporosis-related fracture, which brought the nationwide total cost of treating osteoporotic-related incidents to $14 billion [6].

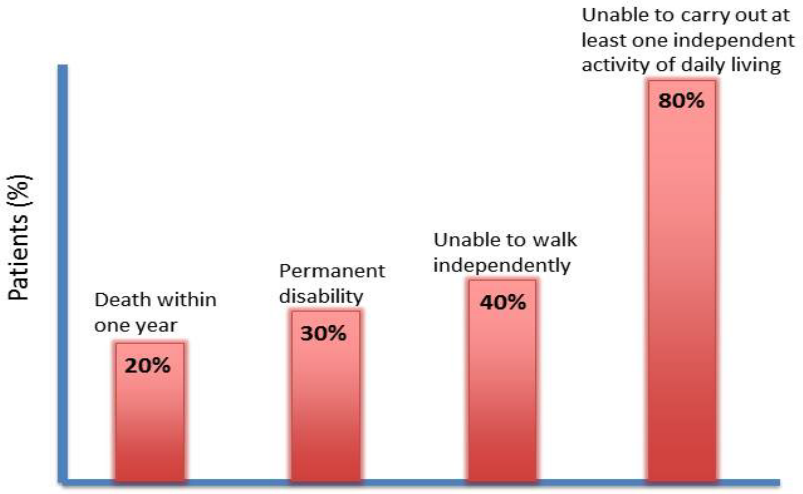

Figure 1.2. Consequences of a hip fracture [2].

As the elder population continues to grow, this multi-billion-dollar problem will continue to compound. Concurrently, the cumulative societal cost of acute and chronic healthcare expenses attributable to osteoporosis and osteoporosis-induced fractures over the next two decades is projected at $474 billion [5]. Individuals with osteoporosis will not only experience a financial burden because of the disease, but many will never return to their pre-injury socioeconomic status [5]. Osteoporotic fractures often result in lost work time or the inability to perform daily tasks, thus decreasing productivity and independence in the home place and the work place (Fig. 1.2).

1.2 Background of Osteoporosis

Osteoporosis is far more common in women. Worldwide, almost 40% of Caucasian women in the United States will experience at least one osteoporosis-related fracture after the age of 50 [7]. A major reason why post-menopausal women are prone to osteoporosis is estrogen levels decline following menopause. This loss of estrogen results in an imbalance of bone resorption and formation (Fig. 1.3). Bone resorption and bone formation are balanced in normal, healthy bone. However, after menopause, bone resorption occurs at a higher rate than bone formation, resulting in lower bone mass, thus

an increased potential risk of fracture [8]. Moreover, as the life expectancy increases, more men are also being diagnosed with osteoporosis. This is likely due to a decrease in sex-steroid production, in addition to age-related bone loss [8].

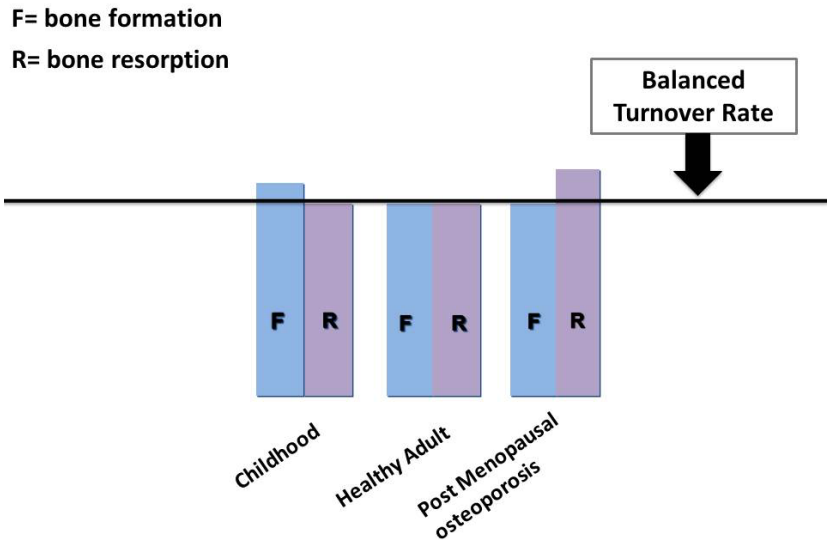

Figure 1.3. The balance of bone resorption and formation changes with osteoporosis.

The diagnosis of osteoporosis is based on a bone mineral density (BMD) measurement. BMD is the amount of mineral per area of bone and is measured via dual-energy x-ray absorptiometry (DXA) [9, 10]. BMD scores are interpreted by comparing the difference, in units of standard deviations, between an individual BMD value for a particular patient's bone to the distribution of BMD values from the same bone in a normal, healthy 30-year-old person of the same gender. This is known as a "t-score" [5, 11-12]. The t- score threshold triggering the diagnosis of osteoporosis, as determined by the World Health Organization, is -2.5[5]. However, it has also been determined that a patient's BMD t-score may not accurately represent risk of fracture as it does not account for all characteristics of bone that influence its ability to resist fracture [9, 10-13, 23]. Here you may wish to add that regardless of t-score a low energy fracture in post-menopausal women, or anyone over the age of 70, may also be a manifestation of bone weakened by osteoporosis and meriting Rx.

Moreover, osteoporosis is often called a "silent disease" as patients may not know that they have osteoporosis until fracture occurs. A decrease in bone density, manifested as increased porosity and thinner cortices in cortical bone, and deteriorated bone structure in trabecular bone characterize this disease. As bone becomes more porous and weaker, there is an increased risk of fragility fracture [3, 4]. The term "fragility fracture" refers to fractures occurring in response to low-energy traumas or normal activities of daily living [5]. While osteoporosis is most commonly associated with decreased bone quantity mass, it is important to note that changes in bone quality also occur with this disease [4].

1.3 Osteoporosis

While osteoporosis is associated with decreased bone mass and increased risk of fragility fracture, there are two different categories of the disease. They are differentiated by etiology, and referred to as primary or secondary osteoporosis [28-30]. Primary osteoporosis is most common in women and is normally age-related or linked to menopause [30]. Secondary osteoporosis occurs equally in men and women and is linked to the presence of other diseases, drugs or physiological conditions [28].

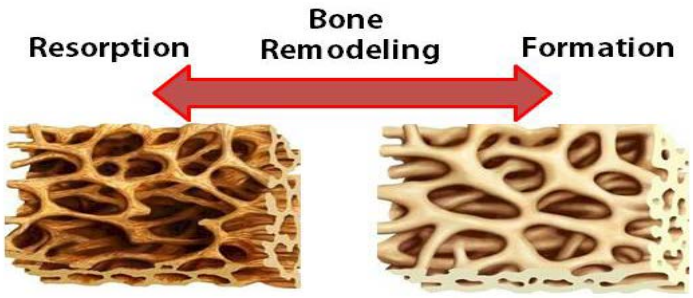

Figure 1.4. Healthy bone exhibits a balance between bone formation and resorption. Diseases, like osteoporosis, can cause this balance to shift towards either accelerated bone resorption or formation.

In women with primary osteoporosis, an estrogen deficiency makes bone more sensitive to parathyroid hormone (PTH), resulting in increased bone resorption [28]. For younger men diagnosed with primary-type I osteoporosis, it is normally the result of medication use or low testosterone levels [8]. In both cases, the balance of bone remodeling is altered to favor resorption, (Fig. 1.4), and results in bone loss. Primary type-II osteoporosis is more common in the elderly [30] and is often the result of age-related vitamin D deficiency, which causes an increase in PTH, again resulting in

accelerated bone resorption [28]. This, in combination with an age-related decrease in bone formation, results in a net loss of bone. Type-II osteoporotic patients are at risk for fracture, just like type-I patients [28-30].

1.4 Consequences of Osteoporosis on Bone Quality and Remodeling

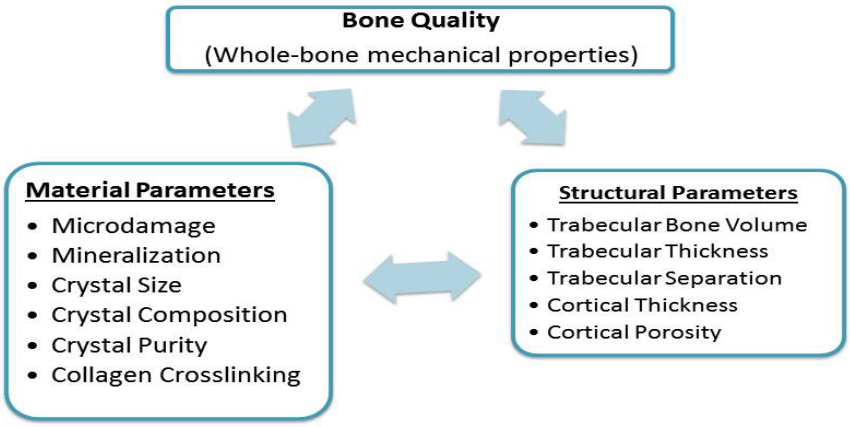

Figure 1.5. The different parameters that influence bone quality.

The concept of bone quality interweaves the mechanical and physiological properties that bone possesses to maintain its structural integrity. Bone quality can further be defined as the ability of bone to withstand loading without significant deformation or failure. Osteoporosis is considered a systemic bone disease and its effects manifest differently in each of the material and structural parameters of bone quality (Fig. 1.5) [4, 14-19].

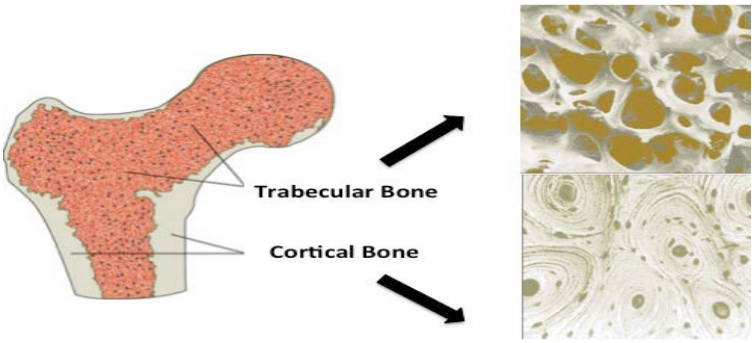

Figure 1.6. The difference in microarchitecture between cortical and trabecular bone. Adapted from [22].

The microarchitecture of bone is considered a structural parameter of bone quality and refers to the structural components of both cortical and trabecular bone. Cortical and trabecular bone are the two different types of human bone and differ distinctly in porosity (Fig 1.6) [20]. The human skeleton has a cortical to trabecular bone ratio of approximately 4:1 [21]. Cortical bone is found in the shafts of long bones and creates an outer shell around trabecular bone. Trabecular bone is much more porous and is primarily found in the ends of long bones, the vertebrae, and the flat bones like the skull and the pelvis [20-21]. Osteoporosis-related bone loss weakens this microarchitecture by increasing the degree of porosity.

Abnormalities in bone turnover and remodeling are responsible for the loss of bone noted in osteoporotic patients, and control both the quantity and quality of bone in the human skeleton [8]. In normal, healthy bone, a balance between bone formation by osteoblasts and resorption by osteoclasts exists. Bone remodeling takes place in a remodeling cavity within an area of bone that needs remodeling. Frost termed this area the Basic Multicellular Unit (BMU), and it contains the osteoclasts, osteoblasts, and the osteocytes [24].

1.5 Mechano-sensitivity of Bone Remodeling

Osteoclasts are responsible for resorbing bone and osteoblasts are responsible for new bone formation. Osteocytes make up the inter-connected network of cells that layer the bone matrix and account for almost 95% of bone cells. They were previously thought to be responsible for sensing changes in the mechanical loading, which directs osteoclastic and osteoblastic actions [25]. However, it has since been determined that all cells within bone are receptive to mechanical loading [25-26].

Wolff's law states that bone remodels as needed to meet its mechanical demands [46]. Reduced loading favors the bone resorption, which decreases bone density and strength. An increased loading favors bone formation, which increases bone density and strength [27].

In healthy trabecular bone, remodeling occurs at the surface and is completed after about 200 days. However, in cases where bone metabolism is altered, such as

diseases like hyperparathyroidism, the remodeling cycle can be as short as 100 days. Remodeling cycles can also last as long as 1,000 days in low bone-turnover states that are induced by other bone metabolic diseases and various pharmaceuticals [27].

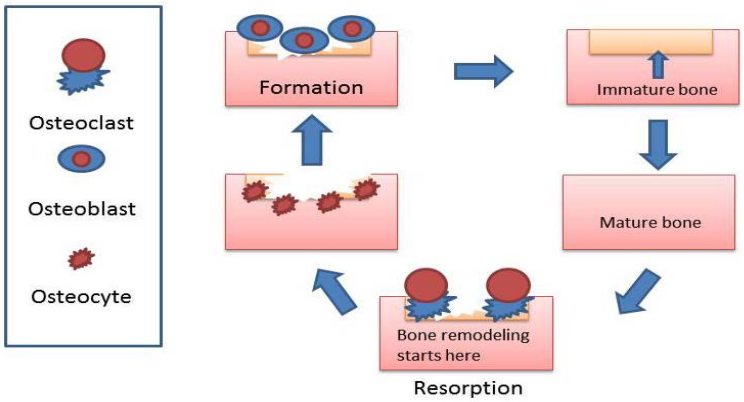

Figure 1.7: The bone remodeling process.

The remodeling cycle (Fig. 1.7) is initiated by osteoclastic resorption, which erodes at the site of remodeling and has a median duration of 30-40 days. Resorption and formation are a tightly coupled sequence where bone formation immediately follows resorption where osteoblasts lay new, un-mineralized bone matrix over the resorption area. Bone formation extends over a period of about 150 days [24]. In a healthy patient, the remodeling area is completely refilled with new bone. Conversely, in diseased states, such as osteoporosis, the main deficiency is that osteoblasts are unable to completely refill the resorption area, resulting in a net loss of bone mass [24].

1.6 Treatments of Osteoporosis

Treatment of osteoporosis begins with two different approaches: increase the rate of bone formation or decrease the rate of bone resorption. Increasing bone formation includes increased calcium consumption, Vitamin D supplements to aid calcium uptake, and regular weight-bearing exercise to stimulate bone formation [3, 31]. Additionally, several therapeutics can be prescribed for patients with osteoporosis.

Hormone Replacement Therapy (HRT) involves administration of estrogen and progesterone and is intended to remedy the estrogen deficiency of post-menopausal women that results in accelerated bone loss [31]. SERMs (selective estrogen-receptor

modulators) are prescribed to stimulate estrogen-like activities within the body. They were developed to have similar effects as HRT, without the potential negative side effects of HRTs [31-32].

Suppression of excess bone resorption is accomplished by using antiresorptive medications and bisphosphonates. Bisphosphonates (Fig. 1.8) are the most prescribed treatment for osteoporosis because of their ability to suppress osteoclast activity, thus slowing bone resorption, and subsequently, loss of bone [33].

1.6.1 Bisphosphonates

Bisphosphonates reduce the risk of vertebral, non-vertebral, and hip fractures [34] and are considered the cornerstone therapy for the onset of osteoporosis [35]. However, it is important to note that bisphosphonates do not build new bone tissue; they suppress bone resorption by inducing osteoclastic apoptosis.

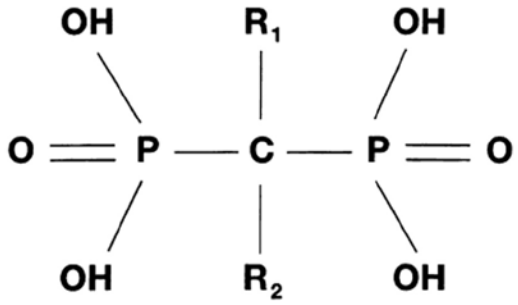

Figure 1.8. The chemical structure of bisphosphonate [27].

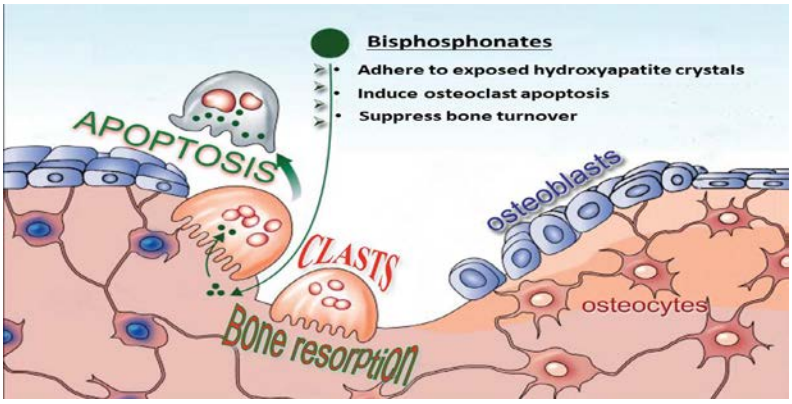

Figure 1.9. Mechanism of bisphosphonates. Adapted from [34].

Bisphosphonates have a high affinity for bone mineral due their chemical structure (Fig. 1.8). They will saturate bone-remodeling sites where hydroxyapatite crystals are exposed [34]. Once bound to the exposed mineral, osteoclasts take up the bisphosphonates and induce osteoclast apoptosis (Fig.1.9) [34]. This is the proposed mechanism by which bisphosphonates inhibit osteoclastic bone resorption in the remodeling space [34].

Sometime after initiation of bisphosphonate treatment, the population of osteoclasts declines, but the existing osteoblasts continues to form new bone matrix. This phenomenon, the *bone-remodeling transient*, is the mechanism believed responsible for the rapid gain in bone mass noted shortly after commencement of bisphosphonate treatment [36, 37]. However, this behavior is only temporary and will cease once the remodeling sites, presently active with osteoblast activity, are filled with newly laid bone matrix [36, 37]. Due to decreased osteoclastic activity, the resorption of bone slows, thus allowing newer bone to mature and increasing the mineral content of bone. Increased mineralization and an initial increase in bone mass led to increases in mechanical strength and fracture resistance and are thought to be the initial beneficial therapeutic effects of bisphosphonate treatment for osteoporosis [36, 37].

Bone resorption and formation, i.e., bone turnover, are tightly coupled. While bisphosphonates suppress bone resorption and reduce bone loss, they also indirectly suppress bone formation [36, 48]. Moreover, bisphosphonates may suppress bone turnover to such an extent that the skeleton might be unable to repair the mechanical loading-induced defects that occur in bone due to normal physiologic activities. These defects are commonly referred to as microdamage. Microdamage is generally considered to have an effect on the clinically relevant mechanical properties of bone [34-35, 38-39, 40-43] and is thus one of the parameters governing bone quality (Fig. 1.5)

Developing a greater understanding of the relationship between bisphosphonate treatment and bone quality is important because the long-term use of bisphosphonates has been linked to atypical fractures. Although the precise cause of these fractures is

unknown, it has been hypothesized that bisphosphonate treatment alters bone quality, and this may be manifested by altering bone microdamage [34-35, 38-39, 40-43].

1.7 Atypical fractures linked to bisphosphonates

Numerous studies have demonstrated the anti-fracture efficacy of 3-5 years of bisphosphonate treatment, but the long-term effects of bisphosphonate treatment are unknown [38, 44-48]. Yet after 3-5 years, longer treatment has been associated with atypical fractures, the majority of which occur in the femur [34-35, 38-39, 40-43]. In 2008, a large case-control study was conducted where a significant correlation was identified between femoral fractures and bisphosphonate use longer than five years in women who had no obvious secondary causes of bone loss [38].

In 2010, the American Society of Bone and Mineral Research established a task force to investigate long-term bisphosphonate use and atypical fractures. This study concluded that the incidence of atypical fractures in relation to bisphosphonate treatment appeared to be very small (.13%), especially when compared with the number of hip, vertebral and other fractures that were prevented because of bisphosphonate treatment [35]. However, preclinical data evaluating the effects of bisphosphonates on collagen cross-linking and maturation, accumulation of microdamage, mineralization, angiogenesis and remodeling provided evidence for an association between atypical fractures and bisphosphonate use [35]. Moreover, observations have been made that suggest that the risk of fracture increases with increasing bisphosphonate treatment duration [35, 38, 40-48].

In 2013, a study [39] proposed the potential mechanism of bisphosphonate-associated atypical fractures. It was hypothesized these fractures are associated with long-term suppression of bone turnover, which is induced by long-term bisphosphonate treatment. At the submicroscopic level of collagen fibrils, it was found that suppressed bone turnover increases the number of non-enzymatic crosslinks, which reduces collagen's plasticity, and consequently contributes to a loss in bone toughness [39, 45]. Suppression of bone turnover increases mineralization [39]. It has been shown that increased mineralization has been associated with decreased bone toughness (Fig. 1.10),

and this, in conjunction with reduced microdamage repair accompanying reduced bone turnover, may partially explain the origin of the increased microcrack density observed in the present study.

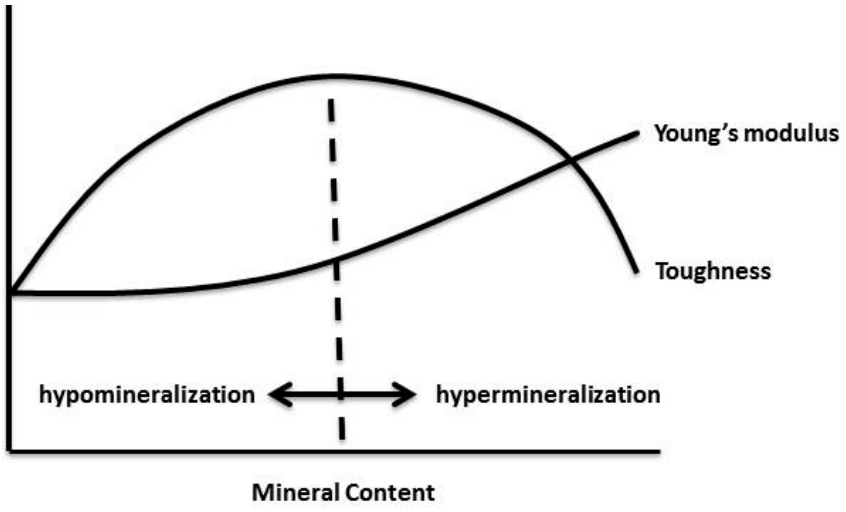

Figure 1.10. The postulated relationships between modulus and toughness versus mineralization (from Wainwright, [47]).

While microdamage is a result of every day normal physiological loading on the human skeleton and a stimulus of bone remodeling, it is unknown if it is a potential mechanism of the observed atypical fractures occurring in women on long-term bisphosphonate treatment. However, bone is a complex composite material with a hierarchy of structural parameters that each responds differently to bisphosphonate treatment and individually contributes to the mechanical properties of the bone.

1.8 Mechanical Properties of Bone

The biomechanical properties of bone (Fig. 1.11) are derived from material and structural attributes. Material-level attributes include the amount, size, and size distribution of bone mineral, the amount of matrix as well as the types and amounts of collagen cross-linking within this matrix, and the degree of material imperfection, commonly measured as microdamage [49-49]. These material level attributes are responsible for bone's intrinsic material properties such as modulus, strength, ductility, and toughness. These material properties are determined independent of bone mass, volume, or geometry [48].

There is a relationship between the material level properties and the structural level properties. Such properties are common in nature and reveal the complex interplay between intrinsic material properties and extrinsic structural properties which attain a desired set of mechanical behaviors for the organ.

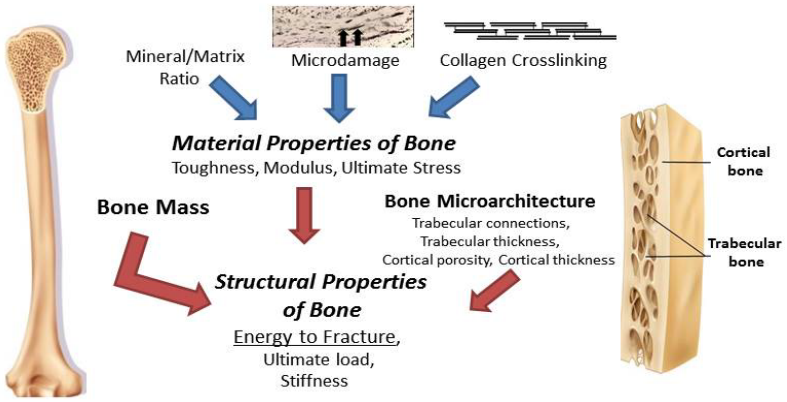

Figure 1.11. The hierarchical levels of bone biomechanical properties. Adapted from [19, 47-48].

1.9 Microdamage in Bone

The presence of microdamage (Fig. 1.12) in bone results in targeted remodeling. [46, 51-53]. Specifically, each time a microcrack is formed, bone remodeling is activated in that area of microdamage [51-52]. Bone remodeling is a tightly coupled sequence that begins with resorption of the damaged area. If resorption is suppressed, perhaps via bisphosphonate treatment, then microdamage-initiated remodeling will be delayed. This means that patients with suppressed turnover, e.g., those on a bisphosphonate regimen will not experience normal remodeling. Suppressed turnover prevents repair of the damaged bone and allows for continued accumulation of microdamage [53, 56].

Figure 1.12. A photograph of a well-defined microcrack (arrows) stained en bloc with basic fuchsin.

Linear microdamage is a material parameter governing bone quality and is defined as microscopic linear cracks 30-200 µm in length [51]. Microdamage occurs because of the natural repetitive mechanical loading that accompanies the activities of daily living [46, 51-52, 56]. Failure of a material from a specific number of loading cycles at a specific stress level (Fig. 1.13) is regarded as its "fatigue limit," [54] where cyclic loading will lead to incremental failure [46, 53]. This is the concept that establishes the mechanical theory that the presence of microdamage may reduce overall bone strength and resistance to fracture [53-55]. However, bone stands alone from any other composite material because it can repair the damage that occurs [46, 51-52, 56].

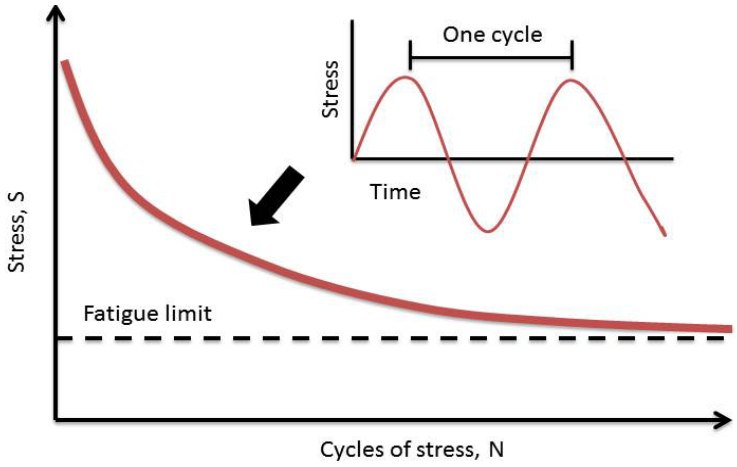

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Figure 1.13. S-N curve demonstrating the fatigue life of bone.

Previous research has established that the presence of microdamage (Fig. 1.12) reduces bone's mechanical strength, stiffness and resistance to fracture; yet there is an inconsistency in the role of microdamage within bone [53-54]. As other researchers have stated, the inconsistency is that the initiation and growth of microdamage initially reduces the risk of fracture because it allows for a dissipation of energy that may have otherwise caused the bone to fracture [52].

1.9.1 Microdamage Initiation and Propagation in Bone

Microdamage is thought to increase resistance to fracture through a mechanism referred to microcrack toughening [60]. Microcrack toughening happens in two stages (Fig. 1.14): 1) formation of the frontal propagation zone and 2) formation of the wake zone [60]. In the frontal propagation zone, microcracks accumulate around the main crack tip (dissipates energy), which reduces the strength of the bone matrix around the tip of the main crack. This eventually reduces the elastic modulus of the bone matrix in front of and surrounding the tip and allows for more crack initiation and propagation [62-64]. The wake zone is the area of increased presence of microcracks left behind in the bone matrix as the main crack continues to propagate. This area has a decreased elastic modulus and allows for more initiation and propagation of microdamage [62-64]. Understanding the mechanisms behind microdamage initiation and propagation is important for better understanding of whole bone fracture and the influence of microdamage on bone quality.

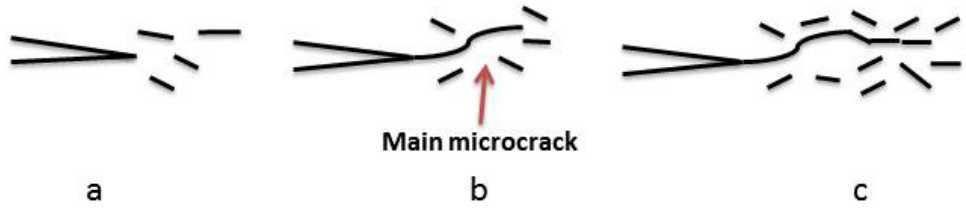


Figure 1.14. Mechanism of toughening. a) Microdamage presence. b) Stage 1: formation of frontal propagation zone occurs as microcracks form to dissipate energy from larger, main microcrack. c) The wake zone of microcracks that were a result of the stress placed on the area due to the larger, propagating microcrack. Adapted from [62-63].

1.10 Aging, Microdamage and the Mechanical Properties of Bone

Microcrack density increases with age [56, 63, 66-68]. However, this microdamage formation is not necessarily pathologic; as it is thought to be the skeleton's natural response to dissipate strain energy and prevent development of fractures at sites subjected to high stress [63]. During mechanical testing, the bone samples of the older subject group had a much shorter fatigue life [63]. Microdamage accumulated more rapidly in an older subject (75 ± 3.9 years), group compared to a younger (61.3 ± 3.1 years) subject group [63].

Aging is associated with reduced turnover. The increased accumulation of microcracks in older bone may be due to reduced turnover because remodeling cannot occur at the rate of microcrack accumulation [66]. With aging comes degradation in stiffness, strength, and fracture toughness [67]. The reduction in stiffness lowers the critical stress level required to initiate a microcrack, and energy required to propagate the crack through the tissue [67-68].

Like the aging process, microdamage also has a negative effect on the mechanical properties of bone [56, 66-73]. A crack density (crack number/ bone area) greater or equal to 1 crack per 1 mm^2 of bone area was associated with reductions of more than 50% in yield and ultimate strength, and a reduction of more than 40% in the modules of the bone samples tested [69]. These studies, along with others [56, 67-73], highlight the multiple effects of microdamage on bone. Despite its role in energy dissipation, it has been concluded that even a small presence of microdamage in human bone can alter its mechanical properties [56, 66-73]. Given the prevalence of osteoporosis, the human and economic consequences of osteoporosis related fractures, the widespread use of bisphosphonates for treating osteoporosis, the hypothesized link between long term bisphosphonate treatment and atypical fractures, and the potential role of bisphosphonate induced bone repair reductions and microdamage accumulation: the goal of the present study was to quantify the relationship between the duration of bisphosphonate treatment and microdamage in human trabecular bone.

Chapter II Rationale

2.1 Effects of Bisphosphonates on Animal Bone

Studies completed in a canine model indicate that microcrack density begins to increase significantly in trabecular bone after only 1 year of bisphosphonate treatment [72-73]. Moreover, bone turnover is suppressed by ~70% after this 1 year of treatment, when compared to a non-treated control group [72]. Additionally, with increased microdamage accumulation, the overall toughness of bone had decreased by 15-20% after this 1 year of treatment [72]. The authors of this study concluded that despite the significant increase in microcrack density, the increases found in bone volume and mineralization were enough to offset any degradation in bone strength that may have resulted from microdamage accumulation [72].

Another study followed, comparing the effects of 1 and 3 years of bisphosphonate treatment. The results showed bone toughness had continued to decline (~ 30%) up to 3 years of treatment and that bone turnover had been suppressed by an additional ~58% when compared to 1-yr treatment animals [73]. Yet, the authors determined that the decline in bone toughness was not due to microcrack density, as the difference between the two groups (1-yr and 3-yr treated) was not statistically different. From these results, the authors hypothesized that microdamage accumulation could still be controlled at only ~30% of normal bone turnover rate. Furthermore, the amount of microdamage was lower at 3 years of treatment because there was a noted increase in bone volume, thus lowering the strain of the trabecular connections, and inversely affecting microcrack density (crack number/bone area) [49, 70, 72-73].

2.2 The Effects of Bisphosphonates on Human Bone

A research effort investigated the difference in microcrack density in trabecular bone between women treated with alendronate for an average duration of 5 years (n=38) against non-treated control group (n=28). After adjustment for covariates (age, BMD, and turnover), the statistical analysis indicated that microcrack density was significantly higher in the treated group [75]. However, despite the significant increase in crack density, mean crack length did not increase with bisphosphonate treatment duration.

These findings correlated with the results of bisphosphonate studies completed in a canine model [49, 70, 76-79].

Additional studies have examined the effect of bisphosphonate treatment duration on not just microdamage, but on other material properties of bone such as collagen cross-linking and degree of mineralization (Fig. 1.10) [78]. It determined that as treatment duration increased, so did bone mineralization. This study attributed these changes as part of what may contribute to bisphosphonates' "anti-fracture efficacy" in osteoporotic patients [78]. However, as the degree of mineralization increases, bone becomes stiffer and will have a lower resistance to fracture. If bone mineralization continues to increase with bisphosphonate treatment, this change may actually impair this anti-fracture efficacy [79]. Evidence available in the literature demonstrates a potential relationship between bisphosphonate treatment duration and bone microdamage. The present study is important because it will provide new information regarding this relationship in human bone treated for longer durations than reported in the literature and do so with large sample sizes, thus permitting regression analyses to quantify the effect of treatment duration and microdamage.

Chapter III Materials and Methods

3.1 Study Design

This cross-sectional study was designed to investigate the effects of varying duration bisphosphonate treatment on microdamage in human bone samples from Caucasian women diagnosed with osteoporosis. Measured microdamage-relevant parameters included: microcrack number, microcrack length, and trabecular bone area. Covariates included subject age, bone mineral density (BMD), bone volume/total volume, (BV/TV), body mass index (BMI), trabecular thickness, and bone turnover. A sample size with a statistical power (β) of 0.8 and a probability level (α) of 0.05 was determined prior to the start of this study.

3.2 University of Kentucky Bone Registry

Bone samples meeting the inclusion criteria, but not violating the exclusion criteria, were identified from electronic data files and physically retrieved from among existing approximately 8,000 samples presently located in the Kentucky Bone Registry maintained by the Division of Nephrology, Bone and Mineral Metabolism at the University of Kentucky Medical Center. Each sample had previously been embedded in poly methyl methacrylate (PMMA) under an established protocol to preserve the integrity of the sample, as well as aid in storage, handling and histological sectioning [76].

3.3 Histological Examination and Analysis

Samples within the registry were obtained from patients who have undergone a routine bone biopsy. Every bone sample undergoes routine histologic and histomorphometric analyses. From these analyses, data for bone mineral density bone volume/total volume (BV/TV), trabecular thickness (TbTh) and activation frequency (Ac.f, bone turnover) was gathered for each sample and utilized in the statistical analysis.

3.4 Inclusion and Exclusion Criteria

Samples meeting the following inclusion criteria were identified from the registry: post-menopausal, osteoporotic, and treated for a continuous duration with oral bisphosphonates. Any patient with a hip or spine t-score lower than -1.5 was considered osteoporotic in this study. Patients were excluded if they had been diagnosed with osteogenesis imperfecta, osteomalacia, or any genetic bone disease, hyperparathyroid

disease, chronic kidney disease, Paget's disease of bone, or any other disease known to alter bone metabolism. In addition, patients were excluded if they had a documented history of drug or alcohol abuse, selective estrogen receptor modulators, sex steroids, teriparatide, or any medications also known to alter bone metabolism.

3.5 Methyl methacrylate Removal

All bone samples that met the inclusion criteria, but not the exclusion criteria, were previously mounted in methyl methacrylate (MMA) for histological examination purposes. Microdamage analyses required that this mounting material be removed. To remove the MMA, the samples were immersed in 2-methoxyethyl acetate at room temperature and under constant stirring until the MMA was completely removed and the surfaces of bone were available to the stains required for microdamage identification.

3.5.1 Staining

Bone samples with MMA removed were then exposed to basic fuchsin to stain the microcracks so that they could be viewed under a microscope and quantified. staining was completed according to an established protocol developed in this laboratory previously [53, 70, 72-76]. The staining was performed *en bloc* with a 1% basic fuchsin solution (JT Baker, B660-03, Phillipsburg, NJ) in a series of graded alcohol solutions (80%, 90%, and 100% EtOH). Each staining step was performed under vacuum at 20 mmHg and constant stirring at room temperature.

1) Time in solution: 48h in 70% EtOH
2) Time in solution: 2h 1% basic fuchsin in 80% EtOH
3) Change solution from step 2
4) Time in solution: 2h 1% basic fuchsin in 80% EtOH
5) Repeat steps 2-4, replacing with 90% EtOH
6) Repeat steps 2-4, replacing with 100% EtOH
7) Rinse in 100% EtOH in order to remove excess staining agent

3.6 Re-embedding of Samples

After staining, each bone sample was placed in a ventilated glass vial with 15 mL of methyl methacrylate monomer and left in a water bath operating at 65° Celsius for 24 hours. After 24 hours in the heated water bath, the monomer solution polymerizes and the bone is re-embedded in MMA. [82-83].

3.7 Cutting of Samples

Stained and re-embedded bone samples were then cut to a thickness of approximately 100 microns using a 150um thick diamond wire saw (Histosaw, DDK, Wilmington, DE) and then histological analyzed.

3.8 Analysis of Samples

A microscope (Axioplan 2 Imaging, Carl Zeiss, Thornwood, NY) was connected to OsteoMeasure software (OsteoMeasureXP V1.01, OsteoMetrics, Decatur, GA) intended for the histologic analysis of tissue. This histomorphometric software was used to quantify the area of bone tissue examined, crack number, and crack length.

Starting at the corner of each section examined, an optical field of 485 µm x 365 µm was examined under 200x magnification. A third party randomized and re-labelled the samples to ensure that the observer who performed all measurements was blinded to bisphosphonate treatment duration.

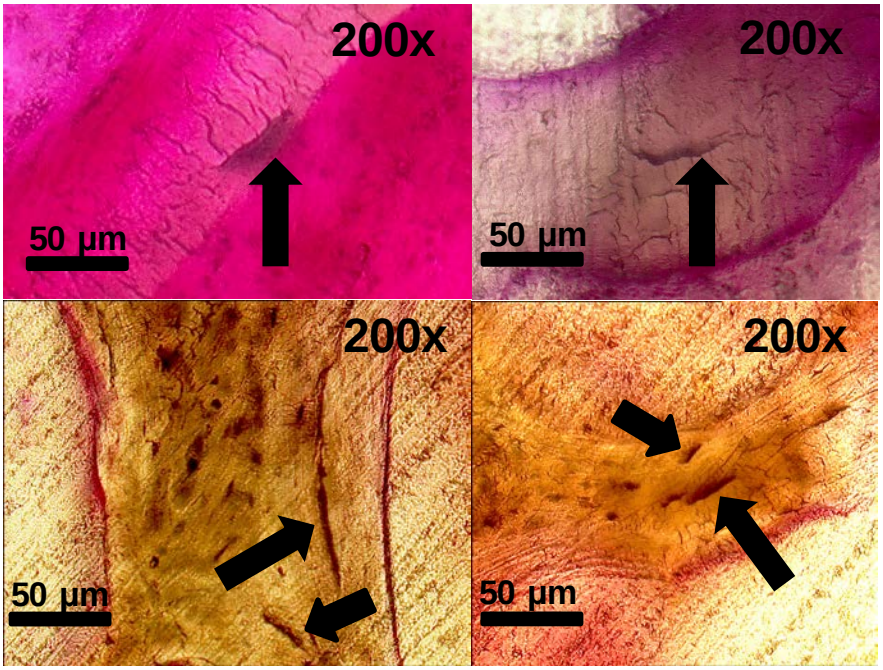


Figure 3.1. Linear microdamage (arrows) in human bone.

Observed microcracks (Fig. 3.1) met all of the following criteria:

- Length between 30 – 200 microns
- Basic fuchsin "halos" surrounding the crack borders.
- Stained through the entire depth of the observed crack.
- Remains visible throughout changes in the focus of the field.

3.9 Microdamage Parameters

Bone microdamage is characterized [46, 51-53, 63-65, 69-77, 80] by the following parameters:

- Bone Area (Br_A) the area of bone examined for microdamage (mm^2).
- Crack number (Cr_N) quantifies the number of cracks per bone sample.
- Crack density (Cr_D) accounts for bone area and quantifies the number of microcracks per mm^2 of bone ($\#/mm^2$).
- Crack length (Cr_L) quantifies the length of a single microcrack (μm).
- Crack surface density (Cr_S.D) quantifies the total length of microcracks in a bone sample per mm^2 of bone area.

The most significant parameter in this study of microdamage is crack density (Cr_D) as it is most representative of microdamage in a bone sample [48-49, 64-65, 69-80].

Microdamage Detection

When analyzing bone samples stained for microdamage, the following techniques were used to identify microcracks that produced *in vivo:*

- A combination of light and fluorescence microscopy [80, 82]
- Altering the depth and focus of the field [82]
- Changing the magnification [82]

When used collectively, these techniques aid in differentiation of actual bone microcracks caused by *in vivo* loading versus scratches or other seeming microdamage attributable to specimen processing [80, 82].

The rationale of these techniques was that pre-existing microcracks have sharp borders and basic fuchsin stains through the depth of the crack [81]. Fluorescence microscopy can be used in the detection of microdamage [81]. A study demonstrated its effectiveness by staining bone samples with the standard en bloc basic fuchsin staining protocol, and quantified microdamage using both light and fluorescent microscopy [81]. It was determined that the two types of microscopy can be used in conjunction to locate microdamage (Fig. 3.2). Using fluorescent microscopy, only microcracks stained with basic fuchsin fluoresced orange against the background field, enabling unstained, or partially stained artefactual cracks to be excluded [81].

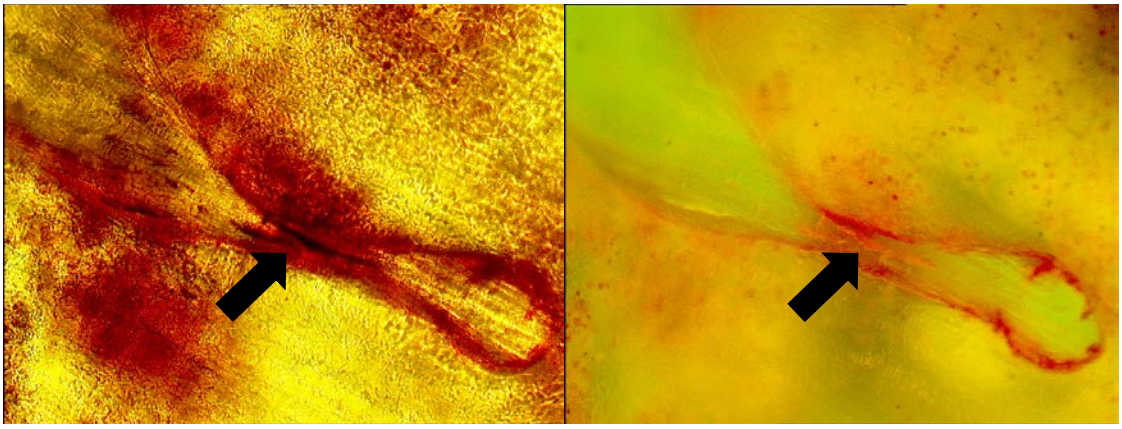


Figure 3.2. Light (left) and fluorescent (right) microscopy can be used in conjunction to define microdamage. Note the presence of the linear microcracks, distinctly defined in both types of microscopy.

3.10 Statistical Analysis

All data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, North Carolina). General regression models were used to relate the response variables (e.g. microcrack number and microcrack length) to duration of bisphosphonate treatment and covariates (e.g. BV/TV, BMD, BMI, trabecular thickness, bone turnover rate, and patient age). Effect of bisphosphonate treatment duration was modeled using linear regression and adjusted for covariates. A $p<0.05$ was considered indicative of significant differences.

Chapter IV Results

4.1 Results

The relationship between increasing microcrack density and bisphosphonate treatment duration was significant, and fit as a linear model (Fig. 4.1). Despite increasing microcrack density, mean length crack did not increase as treatment duration continued (Fig. 4.2). The data were adjusted for BV/TV, BMD, BMI, trabecular thickness, bone turnover rate, and patient age (Table 1). No trend was evident between body mass index, exercising, and increased accumulation of microcracks. Although this analysis is limited due to lack of complete information, it also supports that the increased microcrack densities noted in patients treated with long-term bisphosphonates was associated with treatment duration. Fifty-one samples were included in the analysis. Despite the pre-determined sample size of 71, the current sample size was already large enough reveal a statistically significant relationship.

Table 1: Characteristics of Study Subjects

	Mean	Standard Deviation	Min	Max
Duration (years) (n=51)	7.27	3.23	1	16
Age (years)	63	8.7	41	74
BMI (Body Mass Index)	26.6	5.3	19.2	47.7
BMD hip	-1.8	.74	-2.6	-0.5
BMD spine	-2.0	1.0	-3.2	-0.4
BV/TV	17.04	6.0	4.9	34
Trabecular Thickness (TbTh, μm)	104.9	27.9	55.1	175
Activation Frequency (Ac.f, cycles/yr)	0.2	.12	.02	.46
BP Rx duration (years)*	7.2	3.72	1	16
Crack density ($\mu m/mm^2$)*	3.3	1.98	0.55	7.38
Crack length (μm)	80	24.3	52	169

*$p<0.05$

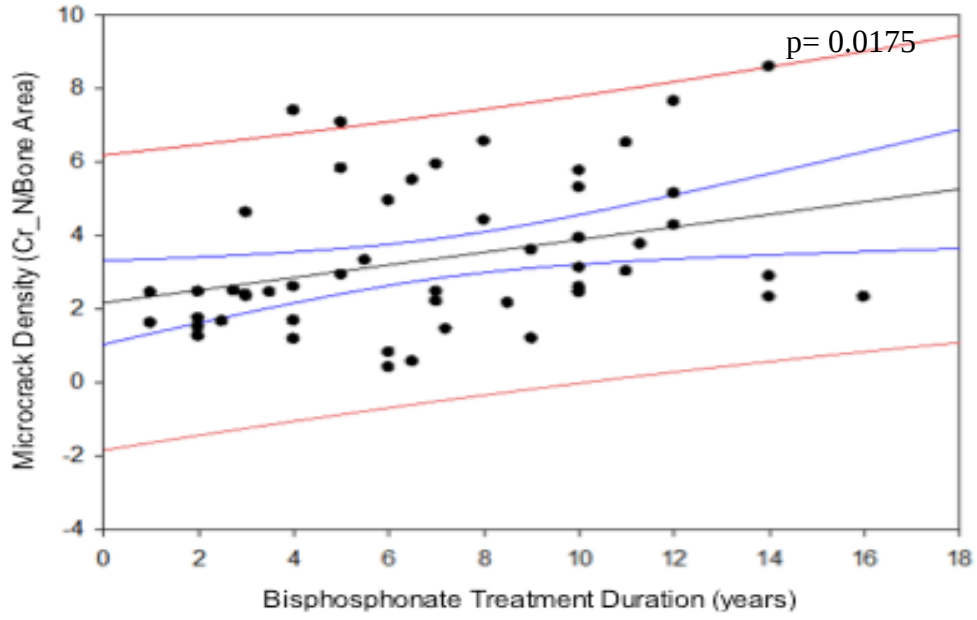


Figure 4.1. Microcrack density and bisphosphonate treatment duration

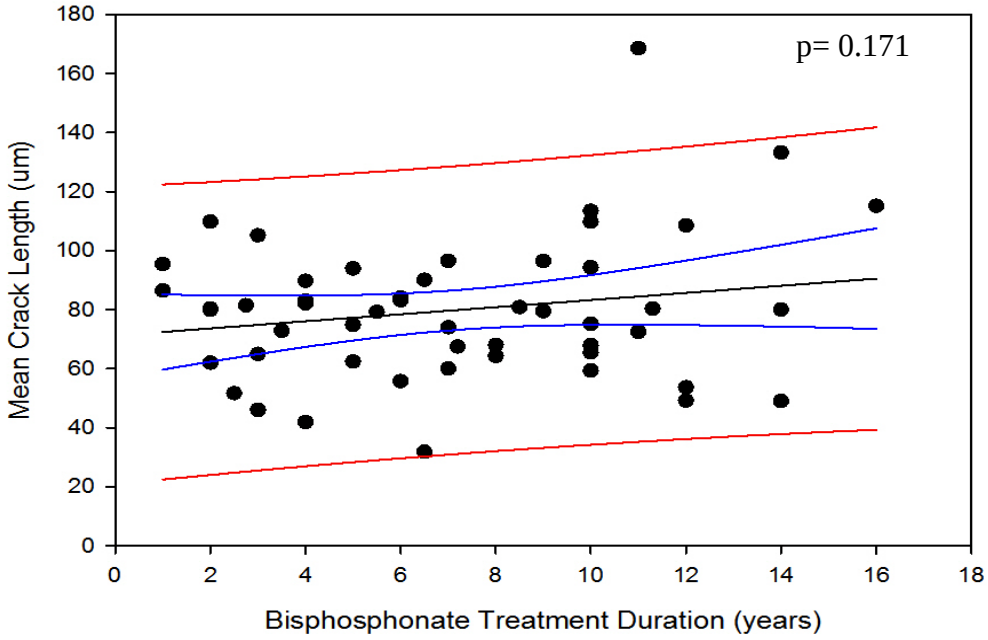


Figure 4.2. Mean crack length and bisphosphonate treatment duration

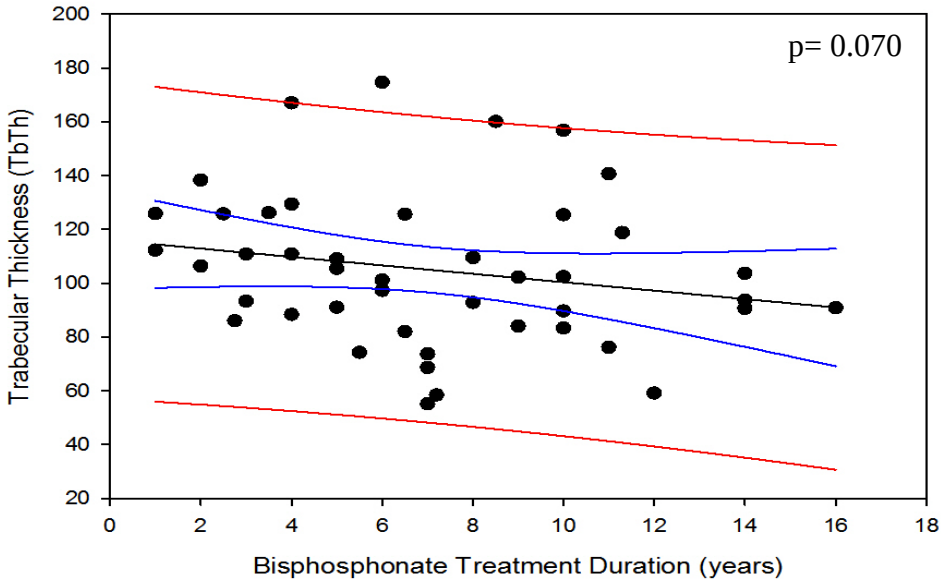


Figure 4.3. Decreasing trabecular thickness and bisphosphonate treatment duration

Chapter V Discussion and Conclusion

5.1 Key findings of this study

The key findings of this study were:

1) microcrack density in human trabecular bone was linearly related to the duration of bisphosphonate treatment,
2) mean crack length was unrelated to the duration of bisphosphonate treatment, and
3) age, BMD, BV/TV, trabecular thickness, bone turnover, and BMI were not significant predictors of increasing microdamage density of samples in this study, and
4) trabecular thickness may be declining as duration of bisphosphonate treatment continues.

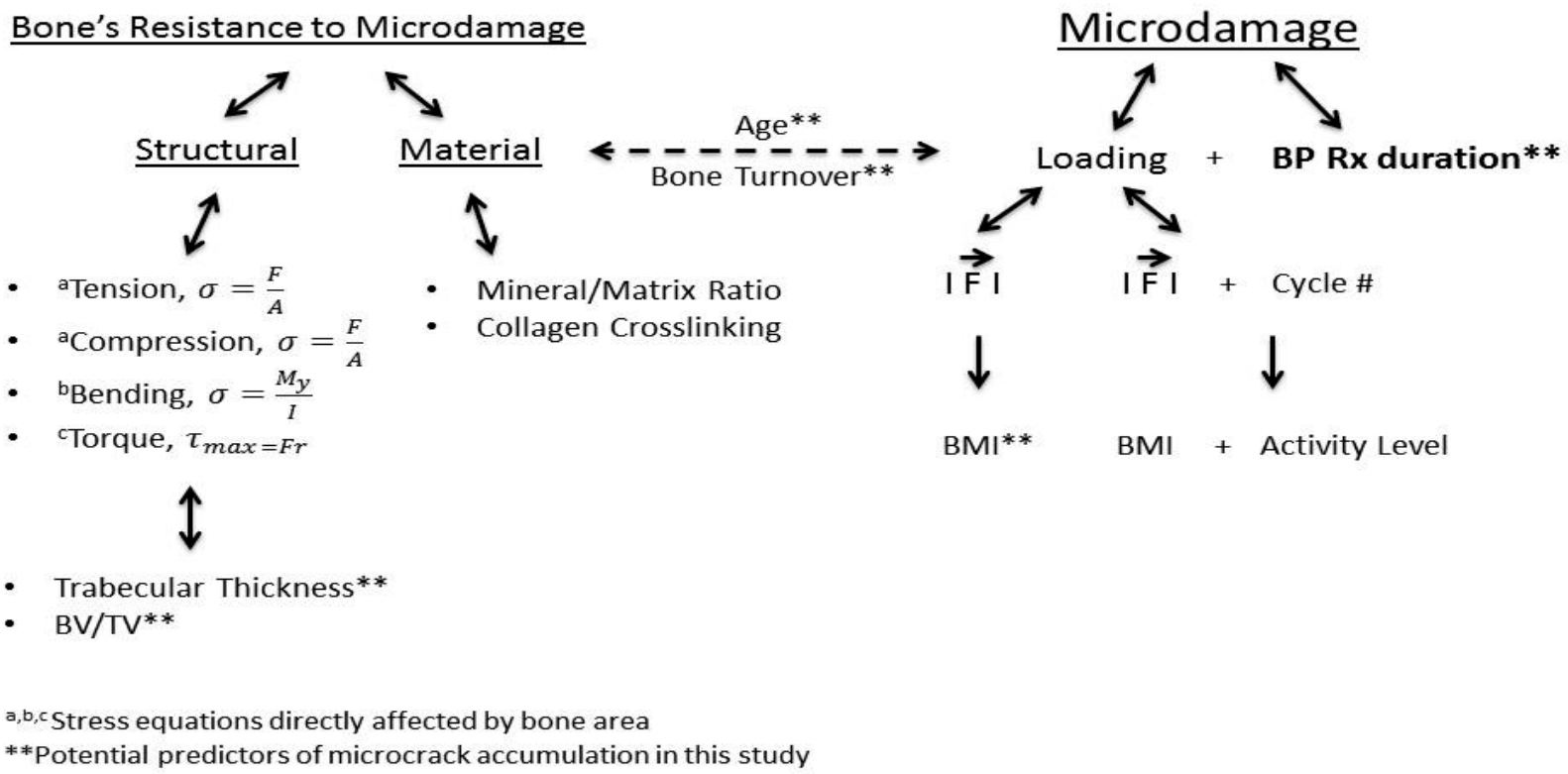


Figure 5.1. The causes and resistance of bone microdamage.

This study provides new insights (Fig. 5.1) into the relationship between bisphosphonate treatment duration and microdamage in bone from women with osteoporosis. Previous studies have studied short-term bisphosphonate treatment durations in animal models and humans, but to the best of our knowledge, this is the first study of the relationship between bone quality and bisphosphonate treatment durations greater than 7 years in human bone. The results of prior bisphosphonate studies in animals and humans [69, 84-87] are consistent with the key findings of the present study.

Age-related skeletal changes are well documented [50, 56, 63]. It has been shown that aging is associated with an increase in microcrack density [63, 66-68]. After noting the relationship between microcrack density and increasing bisphosphonate treatment duration in this study, it was inferred that the effects of aging might have influenced this relationship. Since patient age increases with increasing BP Rx, the observed increase in microcrack density could be related to increasing patient age as well as increasing BP treatment duration. Following adjustment of the data for increasing patient age, the relationship between BP treatment duration and microcrack density changes remained significant. This demonstrates a significant relationship between increasing BP Rx duration and increasing microdamage density without the influence of patient age.

Moreover, age was not the only predictor of increased microcrack density in this study. Reduced bone turnover is the result of bisphosphonate treatment and has been associated with an increase in microcrack accumulation [72-75]. Additionally, it has been determined that bisphosphonate treatment suppresses the targeted remodeling necessary for repairing microdamage [39, 51]. Activation frequency (cycles/year) and bone formation rate (mm^3/mm^2/year) were used to determine bone turnover rate. After the data were adjusted for bone turnover, turnover was found not to be significantly associated with the increased microcrack density noted in this study.

This analysis makes sense, as treatment duration continues and bone turnover is reduced, there will be an increased accumulation of microcracks because of the altered rate of remodeling associated with bisphosphonate treatment. The bisphosphonate-

associated decrease in osteoclast activity (resorption) leads to a decrease in osteoblast activity (formation). For longer durations, continuous bisphosphonate treatment increases the drug concentration so that it directly affects osteoblast activity [76]. It has been determined that at higher concentrations, bisphosphonates promote osteoblast apoptosis or even completely arrests osteoblast growth [76]. The reduction in turnover and bone formation allows for the increase of microcrack accumulation noted in patients treated with long-term bisphosphonate durations.

Change in bone microarchitecture was also a potential predictor for increased microcrack density. Trabecular thickness was analyzed with both microcrack density and treatment duration to determine the relationship between trabecular bone structure, bisphosphonate treatment duration and microcrack density.

Any change in trabecular structural area will result in a change to the amount of stress applied to trabeculae. This is demonstrated in the following equations (Fig. 5.1):

$$\sigma = \frac{F}{A} \quad \text{(a)}$$

Where,

F is force

A is area (*base*height*)

$$\sigma = \frac{M_y}{I} \quad \text{(b)}$$

Where,

M_y is moment (force*distance)

I is moment of interia (*base*height*^{3}/12)

$$\tau_{max=Fr} \quad \text{(c)}$$

Where,

F is force

r is distance from central axis of trabeculae

Therefore, any changes in trabecular structural area (*base, height, and radius*) would have a direct effect the amount of stress acting on the trabeculae. A reduction in trabecular area would result in a greater amount of stress applied; thus inducing increased microcrack accumulation [46, 53]. The statistical analysis, however, revealed that the relationship between trabecular thickness and bisphosphonate treatment duration did not reach significance in this experiment ($p=0.070$). Yet, when the data were fitted as a linear relationship, trabecular thickness looks to be declining as bisphosphonate treatment duration continues (Fig. 4.4). After a power analysis was performed ($\alpha=0.05$, $\beta=0.8$), it was determined that for this relationship to be statistically significant, the sample size must also increase to 71. Potentially, if trabecular thickness was declining as treatment duration increases, the increase in microcrack density could have been the result of structural changes and a subsequent increase in stress on the trabeculae.

It was important to investigate all other aspects the stress equations shown in Figure 5.1 to analyze all potential predictors of increased microcrack accumulation. While it was evident that a decrease in trabecular area would result in higher stress, a direct increase in force would also result in higher stress placed on the trabeculae. The most obvious reason for an increased amount of force on the trabeculae was patient weight. In the United States alone, one in three adults (33%), adolescents or children is either overweight or obese [95]. Patient BMIs were calculated to ensure an accurate representation of weight and examined. A person with a BMI over 25 is considered overweight by national standards [95]. It was calculated that 21 (41%) of the patients had a BMI greater than 25. This equates to 1 in 2.4 people and adequately represents the aforementioned general population ratio. The analysis revealed, however, that there was no significant relationship ($p=0.75$) between BMI and increased microcrack density. This finding further supports that increased microcrack density was associated with bisphosphonate treatment duration.

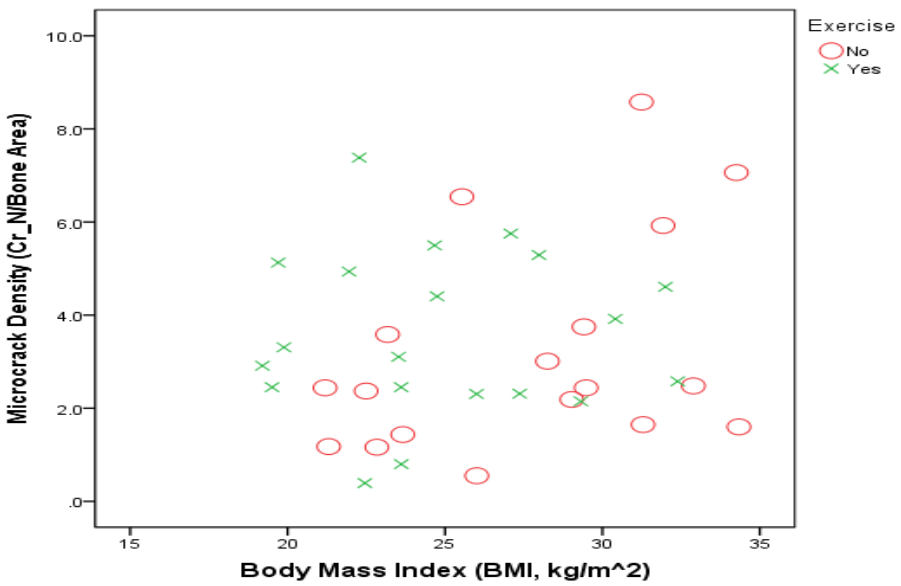


Figure 5.2. Relationships of body mass index, exercise, and microcrack density.

In addition to force, the number of cyclical loads (Fig. 5.2) affects the amount of microdamage present in bone [46, 51-52, 56]. This cyclical loading is the result of daily activity and varied per patient. In this study, patients only indicated if they exercised, but did not give the type or frequency of activity. Due to the vague nature of this information, no interpretation was made if exercise (cyclical loading) played a role in the increased accumulated microdamage noted in this study.

While this study concluded that increased microcrack density was significantly associated with bisphosphonate treatment duration, the intrinsic effects of BP-treatment on the material properties of bone were not examined. Other studies, however, have indicated that a higher incidence and initiation of microcracks exists in bisphosphonate-treated bone. The noted increase in cracks was possibly due to changes in the intrinsic material properties, such as increased collagen cross-linking [87, 89-90, 97], which is associated with reduced bone turnover [35-36, 39, 51, 53, 56].

In fact, a recent study alluded that the potential mechanism behind increased microcrack accumulation is the BP duration-driven increased accumulation of AGEs (advanced glycation end-products) [87]. The bisphosphonate-associated reduced bone turnover is responsible for this increased accumulation of AGEs [87, 89-90, 97]. These

non-enzymatic cross-links act by limiting fibrillar sliding that occurs on a nanoscale level. The limiting effects on fibrillar sliding act to diminish the extent of plastic deformation, therefore reducing intrinsic toughness [89-90] and allowing increased initiation of microdamage within the bone matrix [87].

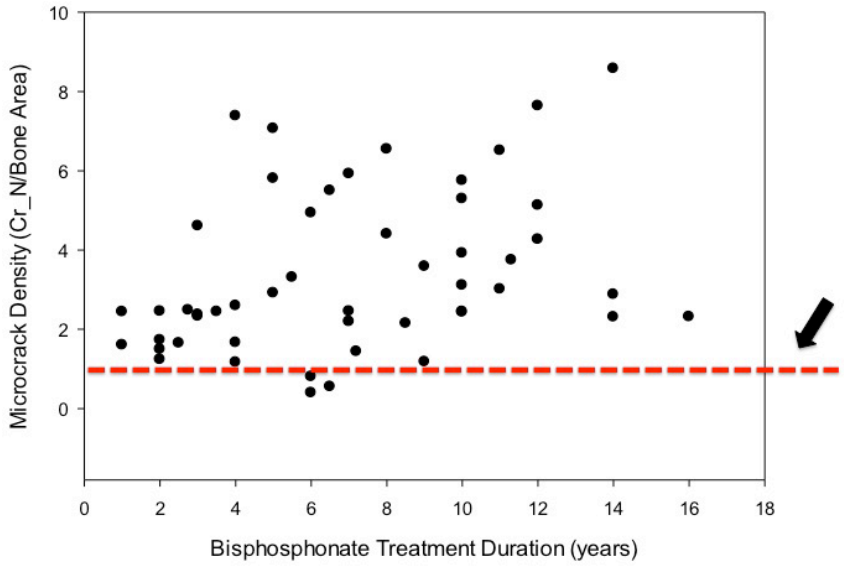


Figure 5.3. Increased microcrack density results in altered bone mechanical properties. In this study, 48 of the 51 (94%) samples had a microcrack density greater than 1 Cr/mm^2.

Furthermore, there are studies that link reduction in bone mechanical properties with bisphosphonate treatment due to microdamage [9, 37, 48-49, 78, 83, 91-95]. Specifically, a study (Fig. 5.3) quantified a microcrack density in trabecular bone (1 crack/sq.mm) that correlated to a 50% reduction in yield and ultimate strength [68]

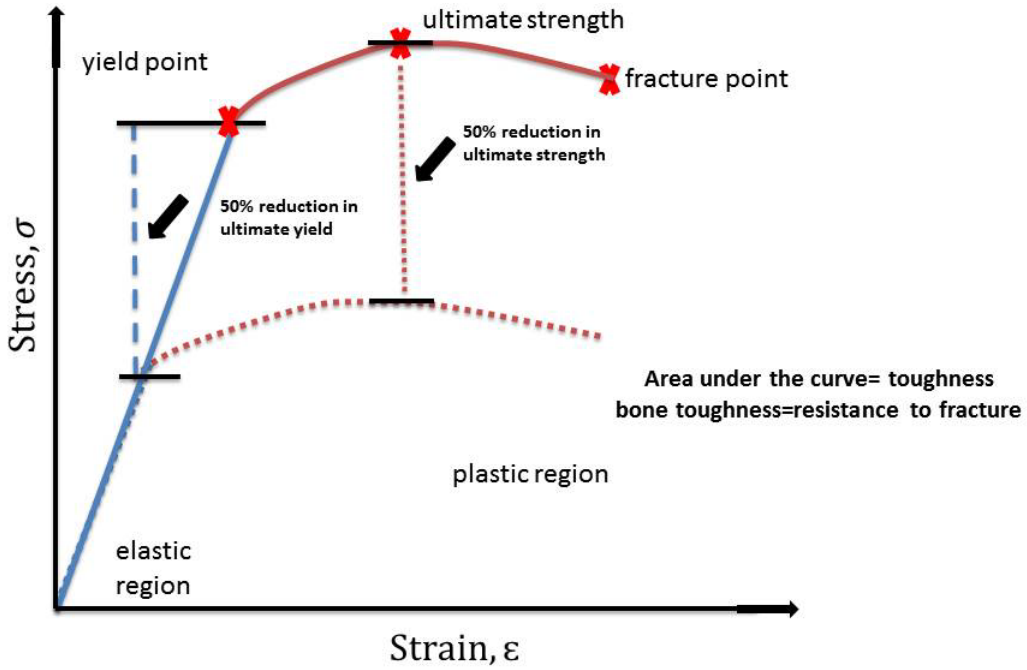


Figure 5.4. Stress-strain curve that illustrates the changes in bone mechanical properties due to microdamage.

With the use of a typical stress-strain curve, it was illustrated how a 50% reduction in yield and ultimate strength affects other mechanical properties of bone (Fig. 5.4). The load bearing capabilities are diminished with reduced strength and yield. In addition, resistance to fracture (bone toughness) is reduced as the area under the curve becomes smaller when yield and strength are lowered because of increased microcrack accumulation.

5.2 Conclusion

In conclusion to this study, it was determined oral long-term bisphosphonate use is associated with a higher amount of bone microdamage in patients the longer they take the drug. Despite a correlation between bisphosphonate treatment and increased microcrack density, however, increasing crack length was not significantly associated with treatment duration in this study. Nevertheless, this data contributes to the characterizing the relationship between microdamage and bisphosphonate treatment duration. Currently, bisphosphonates are an excellent therapy to combat the effects of

osteoporosis and other bone-altering diseases, but studies on how treatment duration affects bone quality need to be continued. Further characterization of the relationships between bone material properties (i.e. microdamage) and structural properties (i.e. fracture toughness) will help to optimize treatment duration.

Chapter VI Importance, Limitations, and Future Work

6.1 Importance

This study presents novel information about the effects of bisphosphonate treatment duration on human bone. By ensuring that the data were analyzed after adjustment for potential predictors (age, turnover, BMD, BMI, BV/TV, TbTh), it allowed the study to isolate the effects of bisphosphonate treatment duration on microcrack density in osteoporotic women.

However, at the current moment, only a few studies have been published [103-104] that associate greater microcrack density with the reported incidences of bisphosphonate-induced atypical fractures. Although it has been shown that microdamage does cause a reduction in mechanical properties of bone, the quantification of a microcrack density that would induce clinically relevant fragility of bone remains unknown and needs to be determined. A previous study, whose findings support this study, determined that under fatigue loading tests bisphosphonate-treated bone contained more microdamage when compared to a non-treated group [97]. The knowledge that bisphosphonate-treated bone has a decreased ability to resist loading-induced microcrack formation [72-73, 75-76, 87, 89-90], and the findings of this study and others all support that bisphosphonate-treated bone treated has a higher occurrence of microdamage. Given the information from other studies indicating that microdamage is related to changes in bone biomechanics, the bisphosphonate treatment duration related changes in microdamage shown herein offer relevant information that may help optimize the treatment of osteoporosis.

6.2 Limitations

As stated previously, only trabecular bone samples were analyzed in this study. To investigate the changes in microcrack density that occur in bone due to bisphosphonate treatment, microdamage should have been quantified in cortical bone. Additionally, this could be a limitation if the sole purpose of this study were to analyze microdamage resulting from bisphosphonate treatment as a mechanism behind atypical fractures; as these fractures primarily occur within cortical bone [34-35, 38-39, 40-43].

An additional limitation to this study was its cross-sectional nature. As such, the data offered insight limited to the date at which the biopsy was performed. An ideal study would have an additional biopsy from each patient before bisphosphonate treatment was initiated to quantify the amount of bone microdamage that existed before treatment. Additionally, patients would have thoroughly recorded their weight and activity levels over their course of treatment. The design of this study, however, best suited the resources available and still succeeded in demonstrating that bisphosphonate treatment duration is significantly associated with the amount of bone microdamage in osteoporotic patients.

6.3 Future Work

The next most logical investigations to pursue that dove-tail with the present findings are, in order of importance, to:

1) evaluate the influence of bone structural parameters on microcrack density. Specifically, this work would determine if a significant relationship exists between trabecular thickness and microcrack density. This study will require additional samples to increase the present sample size to enable a statistically valid determination whether decreasing trabecular thickness is significantly related to increasing microcrack density,
2) determine whether changes in bone composition or structure, i.e., mineral-to-matrix ratio, collagen cross-linking ratio, crystal c-axis length, etc, are significant covariates in the presently established relationship relating bisphosphonate treatment duration and microcrack density,
3) quantify how microcrack density, associated with varying bisphosphonate treatment duration, influences clinically relevant bone mechanical properties, i.e., modulus, yield point, strength, fatigue life, and fracture toughness,
4) bridge the gap in knowledge linking the presently observed microdamage findings with clinically relevant bone load bearing

parameters by using available theories established for classical fracture mechanics.

6.3.1 Griffith's Criterion of Fracture Mechanics

The origin of contemporary fracture mechanics can be traced to the work done during World War I by A.A. Griffith of the Royal Aircraft Establishment in England [61, 99] who sought to investigate the fracture of brittle war materials. Griffith proposed a theoretical analysis of fracture in materials where an applied stress creates a concentrated stress at a defect (microcrack) that is higher than the cohesive strength within the material (bone), and then the defect (or microcrack) will propagate [61]. According to Griffith's criterion, the following two conditions are required for crack initiation and growth:

1) The bonds at the crack tip (cohesive strength) must be stressed to point of failure. The stress at the crack tip is a function of a stress concentration factor, which is governed by the ratio of crack radius to crack length.
2) For crack propagation, the amount of strain energy applied must be greater than or equal to the surface energy of the two new crack surfaces produced [60]. This condition is expressed in the equation shown below.

$$\frac{dU_s}{dc} \geq \frac{dU_\gamma}{dc} \tag{1}$$

Where,

U_s is the strain (applied) energy due to crack propagation

U_γ is the surface energy

dc is the crack length [60, 96].

When a microcrack is formed, two new surfaces are formed as well, raising the surface energy of the material [62]. For a microcrack to propagate, the change in strain energy due to crack extension must be greater than or equal to the surface energy that exists to prevent formation of new cracks [99-102]. Equation 2 represents the Griffith Equation as it can be applied to bone [61]. The stress (σ_c) at which the material (bone) would fracture (microcrack propagation) can be calculated by the following equation.

$$\sigma_c = \left(\frac{2E\gamma_s}{\pi a}\right)^{1/2} \tag{2}$$

Where,

E= elastic modulus of bone

γ_s= specific surface area

a= ½ the length of a crack

The term "fracture toughness" is defined at the ability of bone to resist fracture through changes that occur in the material parameters in the presence of microcracks [58, 90]. Changes that occur in the bone material in front of propagating microcracks are known as "intrinsic toughening mechanisms" [62]. Such structures as cement lines are present at the boundary of osteons work to deflect and blunt crack propagation [64, 95, 97]. Bone's intrinsic resistance to crack propagation can be determined using linear elastic fracture mechanics (LEFM) in terms of fracture toughness, K_c [63]. K_c can be determined *ex vivo* in a bone sample using Equations 3&4. The left side of Figure 6.1 gives a schematic of how a bone sample may be tested to determine its fracture toughness in the presence of microdamage.

$$K_c = \frac{P Y_2}{B W^{0.5}} \quad (3)$$

Where,

P is the applied load

B is the specimen thickness

W is width

Y_2 is a pre-determined shape function [69] that corresponds with *a* and W.

a individual microcrack length

$$Y_2 = \frac{(2+\frac{a}{W})}{\left(1-\frac{a}{W}\right)^{3/2}}\left[\left(0.866 + 4.64\left(\frac{a}{W}\right)\right) - 13.36\left(\frac{a}{W}\right)^2 + 14.72\left(\frac{a}{W}\right)^3 - 5.6\left(\frac{a}{W}\right)^4\right)] \quad (4)$$

Y_2 is a pre-determined shape function [69] that corresponds to individual microcrack length (*a*) and width of the sample.

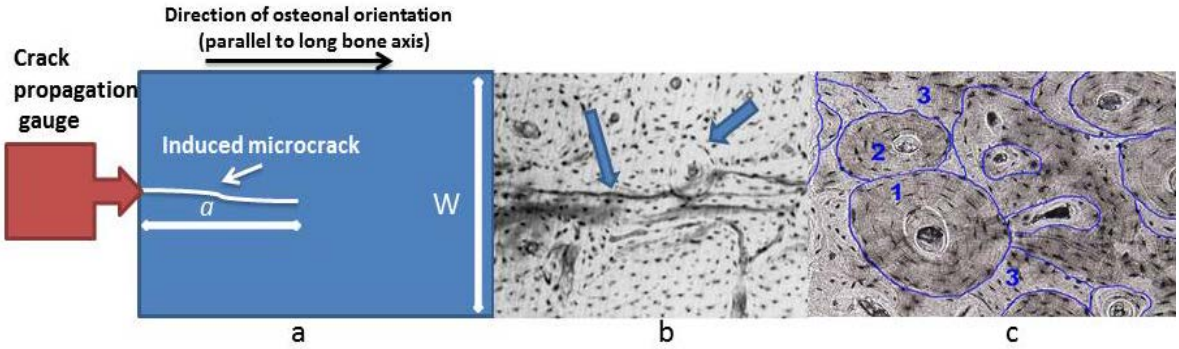
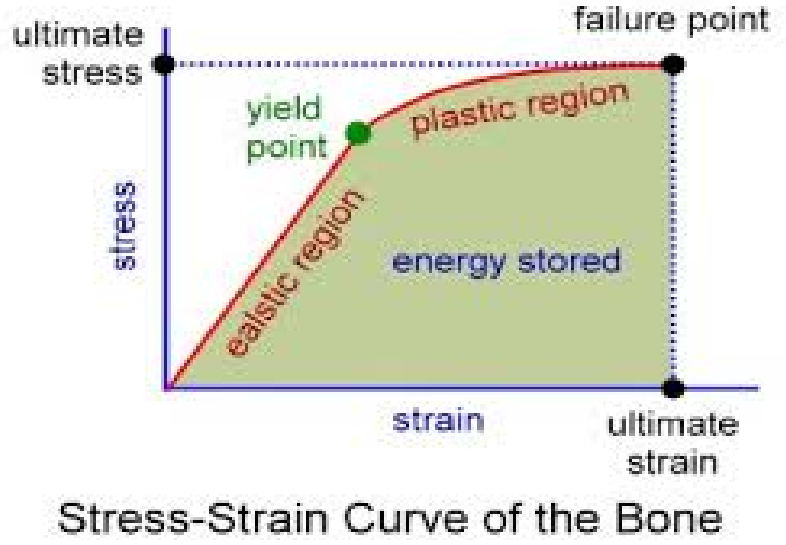


Figure 6.1 a) A schematic diagram for determining the "fracture toughness" of a bone sample. b) Shows an in vivo microcrack being halted by an osteon within the bone matrix. c) A microscopic view of osteons and their cement lines. Adapted from [62].

Appendix A: Mechanical Properties Definitions as related to Bone



Stress-Strain Curve of the Bone

Stress: the loading (force) applied to a known cross-sectional area of an object

Strain: deformation of an object due to stress

Yield strength ("strength"): the stress at which plastic (permanent) deformation begins

Ultimate strength: the maximum amount of stress that can be tolerated by a material

Toughness: amount of energy absorbed by material to a pre-determined deflection

Fracture Toughness (resistance to fracture): amount of energy absorbed before fracture

Elastic Modulus: ratio of stress to strain-measurement of an object's resistance to being elastic (non-permanent) deformation (slope of the line in the stress-strain curve)

Stiffness: rigidity of an object

Sources:
[57, 101, 105, 108]

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