

EXERCISE THERAPY AS TREATMENT FOR POSTMENOPAUSAL
OSTEOPOROSIS IN WOMEN NOT CURRENTLY TAKING HORMONE
REPLACEMENT THERAPY

By

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This document is dedicated to the women who generously gave their time to make this study a reality

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Abstract of Thesis Presented to the Graduate School
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May 2004

Chair: Randy W. Braith

Major Department: Exercise and Sport Sciences

Osteoporosis is a large and growing problem in the United States. Postmenopausal women not taking hormone replacement therapy (HRT) represent a group particularly at risk of osteopenia and osteoporosis. This study was conducted to determine if an intervention of progressive mechanical loading, utilizing specific clinical and commercial variable resistance exercise machines, is an efficacious method for increasing bone mineral density in healthy postmenopausal women not taking hormone replacement therapy.

Forty-six women aged 58.28 ± 5.3 years completed the study. Participants were block randomized depending on whether or not they were taking anti-osteoporotic drugs and were then assigned to one of three exercise groups: treadmill walking, a resistance training program consisting of nine exercise machines (Torso Arm, Chest Press, Seated Row, Overhead Press, Biceps Curl, Triceps Push down, Leg Extension, Leg Curl, and

Abdominal Machine) or a resistance training program consisting of those nine machines plus the Leg Press and MedX Medical Lumbar Extension machine.

All groups were tested at baseline, 18 weeks, and 36 weeks for body composition, total body bone density, and regional bone density in the femur neck and trochanter, and in the lumbar spine. Blood was also drawn at each of these times and stored for later analysis of serum osteocalcin and serum pyridinoline crosslinks, markers of bone formation and resorption respectively.

While we did not see significant gains in bone mineral density (BMD), our findings provide support for the idea that progressive resistance training can attenuate age-related declines in BMD. From the data collected in this study, it appears that resistance training alone may not be a sufficient stimulus to increase bone mineral density in postmenopausal women not taking HRT. For these women, a resistance training program results in significant strength gains but not significant increases in BMD at the hip and spine. While resistance exercise can slow bone loss, pharmacologic intervention may be necessary to achieve increased bone density.

CHAPTER 1 INTRODUCTION

Statement of the Problem

In the United States, it is estimated that one in two postmenopausal women will suffer a fracture engendered by low bone mineral density (BMD)(19). Of the more than 1.5 million fractures that occur each year in postmenopausal women, over 300,000 are fractures of the hip and 700,000 involve the spinal vertebra (55). A recent study suggests that BMD standard deviation (1-SD) below the norm for healthy young females correlates to a 50-100% increase in the risk of later fracture (35). The health care costs of treating osteoporosis-related fractures in the United States is approaching \$17 billion per year (55) and the demographic shift toward an older population clearly indicates this problem will become worse with time. No effective pharmacological therapy to attenuate or reverse bone loss in postmenopausal women is generally accepted. Antiresorptive agents, at best, slow further bone loss but do not have an osteogenic effect. Hormone replacement therapy (HRT) attenuates bone loss but recent evidence indicates the increased risk of stroke, coronary disease, and breast cancer outweighs the anti-osteoporosis benefits of HRT (estrogen + progestin) in some postmenopausal women (30, 80, 90). Treatment with synthetic parathyroid hormone, a treatment with possible efficacy in this population, has not yet become commonplace.

Of all fractures, those involving the hip and vertebra of the axial skeleton have the greatest potential for morbidity and mortality(21). Twenty-four percent of patients die within one year of fracturing a hip and over 40% must be discharged from the hospital to

a nursing home. Follow-up studies have shown that after one year, about one-third of hip fracture patients are still in nursing home (21). Additionally, approximately one-fourth of those surviving a hip fracture will be unable to walk without assistance (19, 56).

Vertebral fractures are also a primary source of mortality and morbidity in postmenopausal women over the age of 50 (19, 56). Postmenopausal women suffer a two- to three-fold increase in risk of vertebral fracture compared to age-matched men (19). Unlike hip fractures where increased mortality is most likely immediately after the fracture, increased mortality associated with vertebral fractures extends well beyond the first year after the fracture (19, 48). Vertebral fractures also cause significant debilitation, increased pain and decreased quality of life. Unlike most bone fractures where pain decreases rapidly as the bone heals itself, vertebral fractures are often characterized by pain that lasts for weeks or months or perhaps even becomes chronic (19). Since approximately 75% of vertebral fractures result not from falls but from routine daily activities, they often cause women to become more sedentary. Women with prior fractures, height loss and/or kyphosis reported greater physical difficulty with routine activities, lower self-esteem and greater fears about their well-being than women with no such conditions (13, 48).

The problem is not just a matter of lost bone but of where the bone is lost from, since trabecular bone is lost at a higher rate than cortical bone. In adults, bone tissue is composed of two basic types: cortical and cancellous. Cancellous bone is found mainly in bones of the axial skeleton, such as the flat and irregular bones that comprise the spinal vertebra, and in the ends of the long bones, such as the femur neck (5). Because of its lattice-like structure, the surface-to-volume ratio of cancellous bone is much greater than

that of cortical bone. This has profound significance for remodeling activity since remodeling takes place at the surface of bones. The greater relative surface area of trabecular bone results in a remodeling rate that is ten times greater than in cortical bone (36). It takes about three months for osteoblasts to fill in the resorption pits created by osteoclasts in only three weeks (33). Over time, this imbalance of resorption and formation accelerates bone loss. Since turnover is greatest within cancellous bone, that makes the vertebrae particularly vulnerable to fracture in postmenopausal osteoporosis, since the vertebrae consist almost entirely of cancellous bone. The hip is also a site of increased fracture vulnerability in postmenopausal osteoporosis because the neck of the femur has no periosteum. Bone apposition primarily takes place at the interface between old bone and the periosteum. However, due to the absence of periosteum bone resorption in the femur neck occurs faster and bone accretion is slower and perhaps more dependent upon external osteogenic stimuli, such as mechanical loading.

Justification for Further Research

There is compelling evidence that osteoporosis is not the inevitable result of aging. Osteoporosis does not simply represent age-related reductions in the responsiveness of bone cells to hormonal influences or the decreased availability of osteoblastic and osteoclastic precursor cells (33, 80). Rather, bone is dynamic and metabolically active tissue that responds to external stresses through the entire life span. Researchers have demonstrated that progressive mechanical loading, which occurs during resistance exercise training regimens, is an effective osteogenic stimulus in adults of any age, when supported by appropriate nutritional conditions. Braith et al. (6, 8, 10) and Mitchell et al. (54) previously demonstrated that a program of resistance exercise training, using specific clinical machines that isolate the axial skeleton, is osteogenic in solid organ

transplant recipients who experience glucocorticoid-induced osteoporosis. What is unclear is whether these same results will be seen in postmenopausal women who are not receiving HRT. Researchers have reported moderate to no BMD gains in younger women undergoing supervised resistance training (1, 43, 89). This may be because the level of exercise activity does not sufficiently exceed their normal daily activities or BMD at study entry was within normal range. However, since women often are less active as they age, a robust resistance training protocol may sufficiently exceed their normal activity thresholds to reach osteogenic levels in postmenopausal women.

Despite the generally accepted axiom that exercise has beneficial effects on bone density, studies have yielded conflicting results. A recent comprehensive meta-analysis of controlled trials (25 articles published between 1966-1996) in pre- and postmenopausal women examined the effects of exercise training specifically on BMD of the lumbar spine and femur neck (89). The overall calculated treatment effect was a modest 0.9% increase in BMD per year, when data from both endurance training and resistance training studies were pooled. When only the controlled and randomized endurance training studies were analyzed, the treatment effect (% change per year of the training group minus % change per year of the control group) was also a modest 0.9% increase in lumbar spine BMD per year (26, 89). However, when only the controlled and randomized resistance training studies were analyzed, the treatment effect (% change per year of the training group minus % change per year of the control group) was a robust 2.6% increase in lumbar spine BMD per year (89). In fact, Notelovitz et al. (59) reported 6.8% increases in lumbar spine BMD per year when utilizing a resistance training regimen similar to the intervention described in the present research protocol. The results

of this meta-analysis clearly suggest that any beneficial effects of an exercise intervention are site-specific and load-dependent. Therefore, the optimal anti-osteoporosis exercise regimen must target and isolate key areas of concern such as the lumbar spine and the femur neck.

There is substantial experimental support for the role of mechanical loading in the regulation of the structure and quantity of bone. However, the mechanisms by which mechanical stimulation leads to new bone formation are not completely understood. Mechanical loading results in small but measurable deformation of the bone; this is expressed as a unit called strain. Animal studies have shown that unusual strain distributions and high strains seem to be particularly osteogenic (42, 71). Application to human subjects implies that strength training with diversity of exercises, instead of endurance training programs such as running, should result in the greatest increase in skeletal density.

There is an emerging body of evidence indicating that osteocytes, embedded within the calcified bony matrix, are involved in the transduction of mechanical stress into a biological response (15, 42, 60, 67). Mechanical loading induces bone deformations that generate cell responses leading to the release of paracrine and autocrine factors. Osteocytes in mechanically stimulated vertebrae, for example, express insulin-like growth factor (IGF-I) shortly after stimulation and levels reach a peak after 6 hours and persist for 1-2 days (15, 42). IGF-I is one of the most abundant growth factors secreted by bone cells. It induces proliferation and differentiation in osteoblastic cells in culture, and it has anabolic actions when administered to animals. The early expression of osteocyte IGF-1 is thought to diffuse along osteocytic channels to bone surfaces where it

participates in the induction of IGF-1 production in bone lining cells (16). Expression of IGF-I in bone surface cells is subsequently followed by bone surface expression of bone matrix proteins, osteocalcin, and type 1 collagen (42). Increases in gene expression are accompanied by the induction of increased numbers of osteoblasts on bone surfaces and increases in mineralizing surfaces, as measured through the use of double tetracycline labels (67).

Research Question

The purpose of this study was to determine whether an intervention of progressive mechanical loading, utilizing specific clinical resistance exercise machines, is an efficacious method of increasing bone mineral density (BMD) in healthy postmenopausal women. We examined whether a nine-month aerobic or nine-month resistance training program elicits significant increases in total body, lumbar, and non-dominant hip BMD. This research assessed how bone density changes as measured by Dual Energy Xray Absorptiometry (DXA) scans and humoral biochemical markers of bone formation and bone resorption in venous blood samples at baseline and after 4.5 and 9.0 months of either aerobic or resistance exercise training.

Research Hypotheses

We hypothesized that:

1. BMD loss will be attenuated in postmenopausal women not taking hormone replacement therapy following a nine-month, supervised progressive resistance training program.
2. A combination of lower and upper body resistance exercises coupled with the use of the MedX lumbar extension and leg press machines will increase hip, lumbar vertebrae and total body BMD more effectively than either an aerobic exercise program or a resistance training program that excludes the low back extension and leg press machines.

3. A progressive resistance training program will increase serum levels of biomarkers (osteocalcin and pyridinoline) indicative of increased bone remodeling and the net change will correlate with increased bone deposition.
4. A supervised nine-month progressive resistance training program will improve skeletal muscular strength in postmenopausal women not taking hormone replacement therapy

To test our hypotheses, we measured regional BMD using DXA at study entry and after 4.5 and 9 months of exercise training. Biochemical markers of bone metabolism (osteocalcin and pyridinoline crosslinks) in human serum were measured at the time of each DXA bone scan. Additionally, DXA scans will be offered annually for a follow-up period of 5 years after the exercise intervention ends in an attempt to assess the long-term effects of the exercise programs on BMD and bone fracture rates.

CHAPTER 2 BONE BIOLOGY AND METABOLISM

Introduction

In the United States, it is estimated that one in two postmenopausal women will suffer a fracture engendered by low bone mineral density (1, 19). Unfortunately, there are no outward symptoms to warn a person they are losing bone density often until it is too late. Loss of height, bones breaking under low- or no-load conditions or the onset of kyphosis are indications bone density has already decreased significantly. Of the more than 1.5 million fractures that occur each year in postmenopausal women, over 300,000 are fractures of the hip and 700,000 involve the spinal vertebra (69). A BMD even just 1-standard deviation below the norm for healthy young women correlates to increased risk of later fracture and the risk rises exponentially as BMD deviates from norms (Figure 2-1) (35, 65).

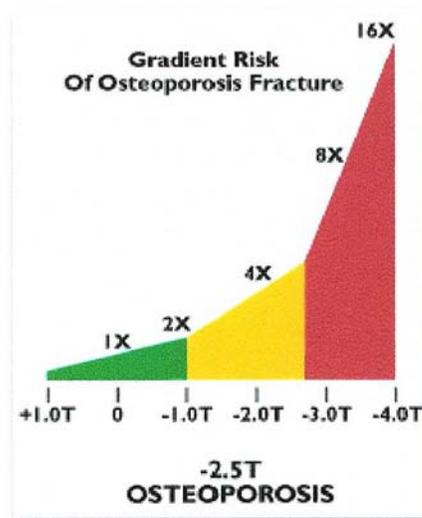


Figure 2-1. Relationship between T-Score and Fracture Risk (29)

These statistics argue the need for an improved understanding of bone biology so researchers and clinicians can develop more effective treatments for declining bone density, whether it occurs as a function of aging and disease or whether it is artificially induced by medications. The starting place is to understand bone structure and bone metabolism. This chapter will review the structure of bone, theories of bone remodeling and the role of estrogen and exercise in moderating bone loss.

Bone is a complex tissue. It provides structure and protection, serves as a repository for some key minerals, contains hematopoietic tissue in its marrow and carries out its own growth and repair processes. Bone responds to both external and internal stimuli and adjusts its composition in response to mechanical stress. These activities are all part of bone metabolism, the physiological process by which the bone functions as living, adaptable tissue.

The Structure of Bone

Bone tissue in adults is of two basic types: cortical (or compact) and cancellous (also called spongy or trabecular bone). Cortical bone is the hard outer layer of bone. It is denser than cancellous bone, which is found closer to the bone marrow (Figure 2-2).

Cancellous tissue is mainly found in bones of the axial skeleton, in flat and irregular bones, and in the ends of the long bones (5). This type of bone experiences deformation when it is loaded and is better able to bear loads without becoming damaged (68). The lattice-like structure of cancellous bone means its surface-to-volume ratio is much higher than that of cortical bone. Since remodeling takes place at the surface of bones, the greater relative surface area of cancellous bone means remodeling takes place there at a rate ten times greater than it does in cortical bone (5, 36).

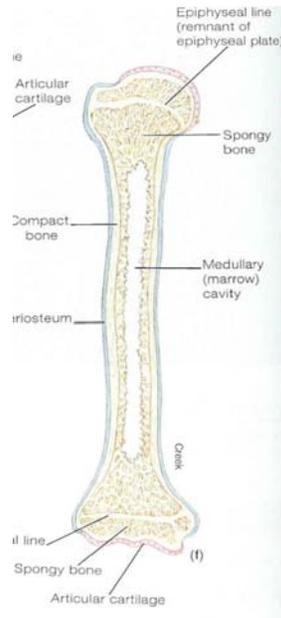


Figure 2-2. Cortical and Cancellous Bone (85)

Thus, when there is an imbalance leading to more resorption than formation, the effects will be most apparent in cancellous bone, i.e., in irregular bone (such as the vertebrae) and the ends of long bones. The difference in metabolic rates between bone types is striking. Bone turnover in the cortical bone of the radius is as low as 2% per year, whereas trabecular bone in the ilium can be as high as 50%. Turnover in vertebral cancellous bone is somewhat less than in the ilium, but significantly greater than the 5% annual turnover found in the femur neck (57).

Periosteum, a fibrous connective tissue, covers the outermost surface of the cortical bone. The periosteum is not present, however, where tendons or ligaments insert into a bone, on bone regions lined with articular cartilage, or on the neck of the femur. The bone contiguous with the periosteum is the site of most bone formation, so areas lacking periosteum, such as the femur neck, experience apposition at lower rates. By volume, cortical bone makes up about 80% of the adult skeleton. The remaining 20% is the more changeable cancellous bone. In contrast to the hard and only slightly porous cortical

bone, cancellous bone is a complex three-dimensional network of curved plates and rods 100 to 150 micrometers thick and is located inside cortical bone. Cancellous bone is made up of a lattice of large plates and rods collectively called trabeculae (see Figure 2-3). The endosteal side of the bone is continuous with cancellous bone at the inner portion of the bone near the medullary cavity (5).

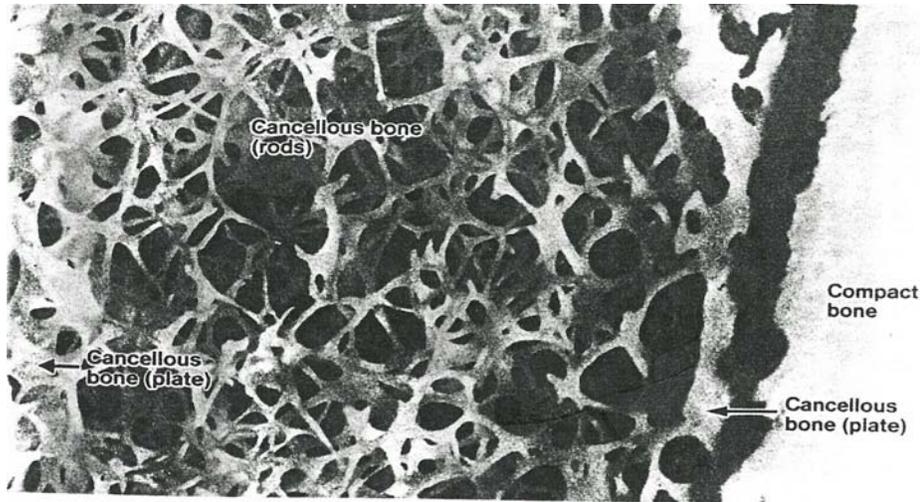


Figure 2-3. Electron Microscope Image of Tibia. Shows cortical and cancellous bone with Trabeculae.(5)

Bones do not contain cortical and cancellous bone tissue uniformly. A typical long bone has three regions that vary in composition. The diaphysis, the shaft of the bone, is comprised mainly of cortical bone. Cancellous bone lies beneath the cortical layer but is much less by volume and less remodeling takes place here than at the ends of bones. The ends of the long bones, known as the epiphyses, are to a large degree cancellous bone, as is the metaphyses, the conical section of bone connecting the epiphysis and diaphysis (Figure 2-4).

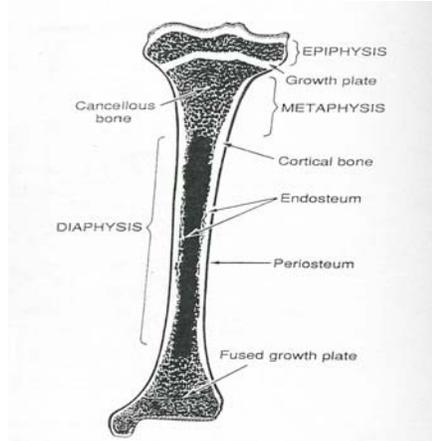


Figure 2-4. Structure of a Typical Long Bone (5)

Figure 2-5 shows that the head and neck of the femur are largely cancellous bone.

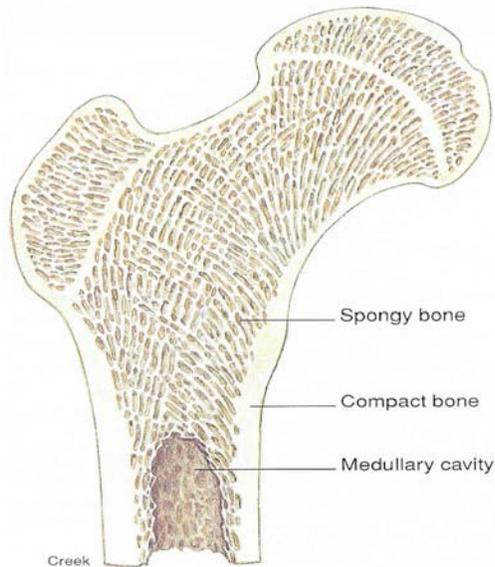


Figure 2-5. Cancellous Bone of the Proximal Femur (85)

The femur's architecture and lack of periosteum suggest why its neck is at increased risk of fracture since it not only has no periosteum to aid bone apposition but it also has a high percentage of cancellous bone. An example of the high level of remodeling in long bones is shown in Figure 2-6 (picture courtesy of Dr. Tom Wronski).

Depicted on the left is a normal rat humerus while the picture on the right shows the bone loss that accompanies surgical inducement of menopause.



Figure 2-6. Rat Humerus. Left picture is of a normal rat humerus. On the right is a rat humerus three months following ovariectomy

While the appendicular skeleton has many long bones and a few irregular ones, the axial skeleton has many irregular bones such as the vertebrae. The irregular bones consist primarily of cancellous bone. Since the axial skeleton has a great deal of the more metabolically active cancellous bone tissue it is not surprising that it experiences greater bone density loss than the appendicular skeleton. Figure 2-7 shows normal and osteoporotic human vertebrae (picture courtesy of Dr. Tom Wronski).

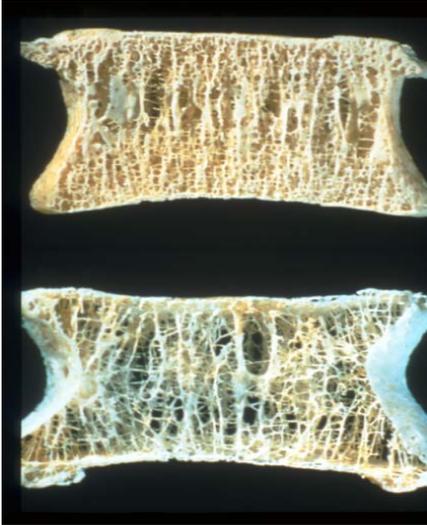


Figure 2-7. Human Vertebrae. Top: normal young adult human vertebrae bottom: Osteoporotic vertebrae of a 75 year old women.

Figure 2-8 shows how osteoporotic vertebra can result in compression fractures leading to the commonly seen dowager's hump, the common name for kyphosis.



Figure 2-8. Magnetic Resonance Imagery of a Thoracic Vertebra Compression Fracture (47).

Cancellous bone is made up of plates of bone tissue and loss results in a gradual shift from plates to rods as the dominant structural element (62).

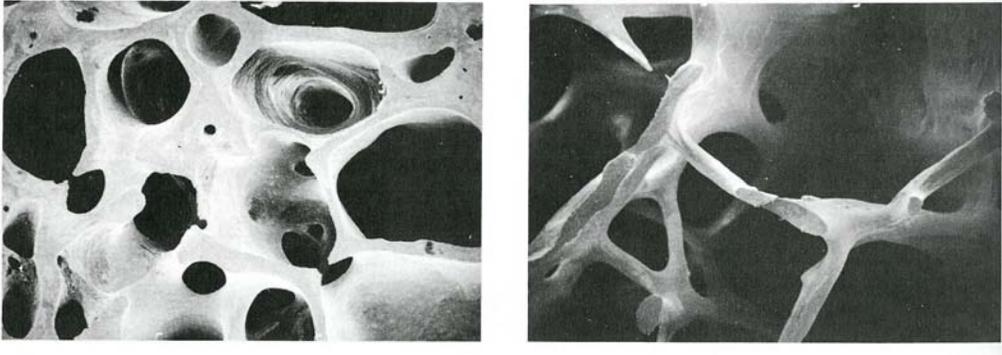


Figure 2-9. Electron Microscope Image: Biopsy of normal (left) and osteoporotic (right) Iliac Crest (85)

This contributes to increased fragility of the bone, since spaces within the bone increase as the “struts” connecting one section to another disappear. Electron microscopy of an older person’s bones show wider spaces and fewer structural connections (figure 2-9). This has important implications for bone strength since trabecular struts, once lost, cannot be replaced (62). Researchers believe that trabecular disappearance occurs when osteoclasts erode a cavity too deeply or when the osteoblasts are unable to lay down a sufficient amount of replacement bone (62).

Bone changes as it is deposited and matures. Cancellous and cortical bone are found in two forms: woven or lamellar. The developing embryo, as well as fracture sites, has mostly woven bone. Woven bone gets its name from the fact that its collagenous fibers form irregular or woven bundles. After birth, the more finely bundled lamellar bone replaces woven bone through the remodeling process (5). The body also deposits new bone in woven form. The woven bone is a matrix of coarse collagen fibers interwoven with osteocytes. It is less organized and shorter-lived than lamellar bone, which replaces it over time. Lamellar bone, as the name implies, consists of a number of layers (the lamellae). Each lamella contains fibers which run generally in the same direction, although successive lamellar layers can have their fibers oriented up to 90

degrees off axis. Both cortical and cancellous bone have lamellae. Within the lamellae there are small cavities called lacunae, which are interconnected by tubular canals called canaliculi. These are found throughout woven and lamellar bone in both cortical and cancellous bone samples. The cells trapped within the lacunae are osteocytes and they have long cytoplasmic processes that extend between the lacunae via the canaliculi (Figure 2-10). This allows contact between osteocytes in separate lacunae.

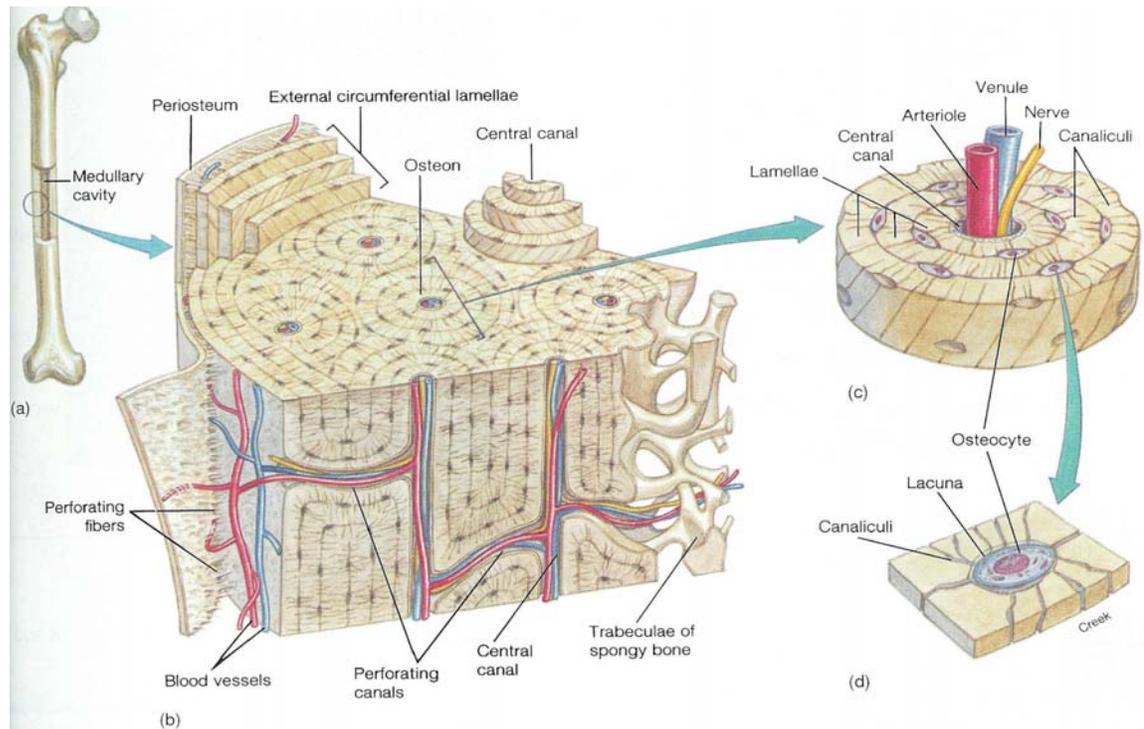


Figure 2-10. Internal Structure of Typical Long Bone (85)

The lamellae of cortical bone make up the basic structural unit of bone, the osteon or Haversian system. Osteons make up about two-thirds of cortical bone volume, although this proportion declines with age. The remaining one-third is interstitial bone made up of remnants of previous generations of osteons and lamellae. The osteon is cylindrical and approximately 200-250 micrometers in diameter and roughly parallels the long axis of the bone. Each has a central canal about 40-50 micrometers in diameter.

These canals connect to each other via transverse Volkmann's canals and house blood vessels, lymphatics, nerves and loose connective. The wall of a typical osteon is comprised of 20-30 concentric lamellae (5).

The major components of cortical and cancellous bone are type 1 collagen, water, hydroxyapatite mineral (calcium salt), and small amounts of proteoglycans and noncollagenous proteins. Type 1 collagen is a structural protein found mainly in bone and tendons. Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, makes up virtually all of the mineral in bone. The individual crystals take the form of rods or plates and result from mineralization. This represents the major storehouse for the body's calcium. Calcium is taken in or released based on fluctuating plasma calcium levels and the presence of the major calcium regulating hormones, PTH and calcitonin. The ground substance (matrix) of bone consists of the proteoglycans decoran and biglycan. Although researchers do not entirely understand the role of proteoglycans they seem to affect collagen fibril assembly and may influence the location or rate of bone mineralization. Osteoblasts also secrete noncollagenous proteins such as osteocalcin which assists in mineralization of new bone. Researchers also believe that osteocalcin is a chemoattractant for bone cells (49, 50).

Cellular Components of Bone

Bone cells include osteoclasts, osteoblasts, osteocytes, and bone-lining cells. Each cell type is critical to bone remodeling. They operate in balance generally, although aging, some disease conditions and certain drugs can alter the balance. These cells have complex means of communication that controls their actions and interactions. Bone remodeling requires the concerted work of each of these cell types. There is ample opportunity for the balance in bone metabolism to shift toward more resorption than

deposition since the osteoclast is able to dissolve in one day what it takes the osteoblast several days to fill in.

Osteoclasts

Osteoclasts are large, multinucleated (2 to 50 nuclei) cells associated with bone resorption. Osteoclasts come from hematopoietic stem cells in the bone marrow and travel via the circulatory (or perhaps the lymphatic system). These progenitor cells settle in the stromal mesenchyme in and around bone tissue near periosteum and the internal surface of bones (often called the endosteum).

Mature osteoclasts (see Figure 2-11) are the cells responsible for bone resorption, where bone is broken down and the calcium within liberated. Osteoclasts travel to resorption sites via the bloodstream and are found in cavities on the bone's surface area, which they themselves form, called resorption pits or Howship's lacunae. Interestingly, although associated with resorption, osteoclasts have no receptors for PTH, the main endogenous mediator of bone breakdown (84). Instead, osteoclasts have receptors for calcitonin, a hormone that stimulates bone formation.

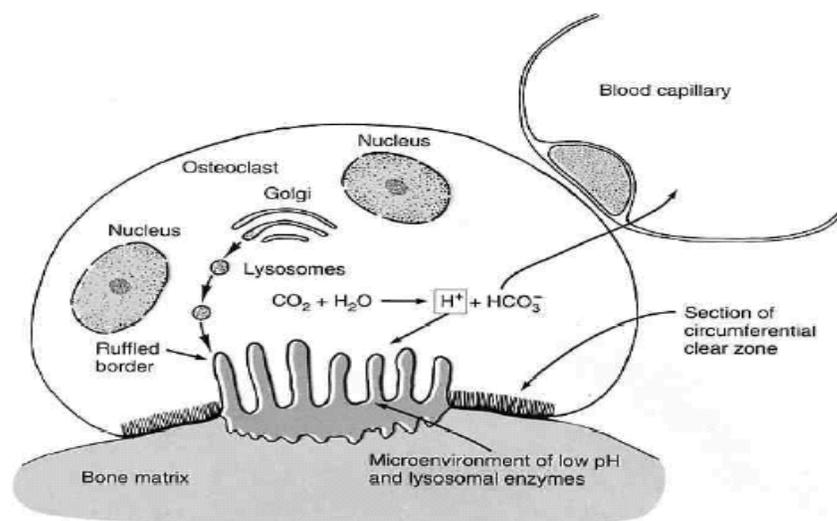


Figure 2-11 An Osteoclast (23)

Osteoblasts

Osteoblast precursors are located near bone surfaces of the periosteum, the endosteum, and the adjacent marrow stroma. They form from local, undifferentiated intraskeletal mesenchymal cells capable of mitosis. Mature osteoblasts are bone-forming cells that typically reside on the bone's surface or in the marrow where they secrete the unmineralized matrix, called osteoid, during the bone formation process. They also participate in calcification of bone and regulate the movement of calcium and phosphate into and out of the bone. These cells are normally cuboidal in shape. While they contain an abundance of endoplasmic reticulum, they rarely undergo mitosis. Osteoblasts produce and secrete a number of substances important to bone metabolism including type I collagen, non-collagenous matrix protein, osteocalcin, osteonectin, several matrix proteins, growth factors, prostaglandins E₁ and I₂. Osteoblasts secrete osteoid at the rate of about 1 micrometer per day. This means that either the lifespan of the osteoblast is quite long or that multiple generations of osteoblasts are involved in refilling a given resorption pit since these pits can be quite deep (49, 50). Although osteoblasts are most noted for bone formation, they may also help control bone resorption since they have receptors for PTH.

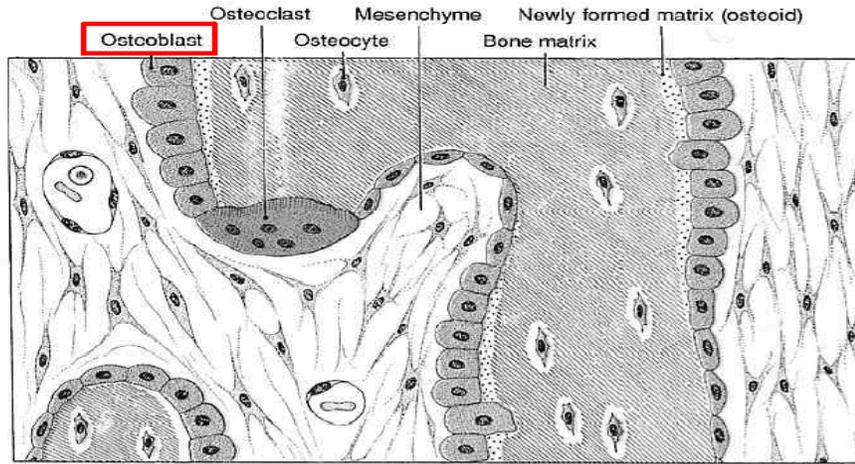


Figure 2-12. Cells Involved in Bone Remodeling (23)

The lifecycle of an osteoblast includes creation from a precursor cell, differentiation, and participation in matrix formation. Following this, they reenter into the preosteoblast pool, transform into bone-lining cells, get buried as osteocytes, or die (5).

Osteocytes

Osteoblasts which become trapped in the osteoid they secrete are called osteocytes and are the principal cells of fully formed bone. Each lacunae contains only a single osteocyte. These cells maintain contact with each other and with bone-lining cells via slender processes that reach through the canaliculi of the bone at the gap junctions. There are gap junctions between adjacent bone-lining cells and between bone-lining cells and osteocytes. Osteocytes are involved in detecting and repairing microfractures and the cell signaling that directs remodeling (5, 49, 50). They may also be involved in storing mineral ions after a meal rich in calcium and in transporting minerals from deeper skeletal reservoirs to the extracellular fluid compartment after resorption (5).

Bone Lining Cells

The final major bone cell type is the bone-lining cell. These are long, flat cells that cover quiescent (or resting) bone surfaces where bone is neither being resorbed nor formed. Like osteocytes, bone lining cells originate from osteoblasts. They differ from osteocytes, however, in that newly formed bone does not bury them. As bone formation ends, bone lining cells remain on the newly formed bone surface. They can still communicate with osteocytes and each other through gap junctions. Researchers have shown these cells retain their PTH receptors. The bone lining cells assist the osteocytes in moving mineral in and out of the bone and may also play a role in sensing mechanical strain on bone (59). There are about 19 of these cells per millimeter of bone surface.

Bone Remodeling

Bone is a dynamic tissue that constantly changes through the life cycle. Even the bone present at birth is generally resorbed and replaced by the time a child is 2-3 years old (5). Except for endochondrial ossification at the epiphyses, new bone grows by apposition only; that is, by laying down new bone on the surfaces of existing bone. Continual remodeling of bone insures it maintains strength and structural integrity so long as there is a balance between resorption and deposition. In childhood, there is generally a positive balance in bone remodeling, meaning that more new bone tissue is formed than is removed. This does not imply, however, that bone breakdown slows in favor of formation. In fact, researchers have found that increased bone resorption accompanies new bone formation both in children and adults (5). In the aggregate, what determines whether more bone is being formed or resorbed is the relative amount of each.

Bone remodeling is the term used to describe the processes required to resorb old bone and deposit new bone at a site. Bone remodeling is an on-going “housekeeping”

activity of healthy bone. Even in the absence of external stimuli there will be some remodeling activity. Old bone gets resorbed, and new matrix is laid down in its place. When there is sufficient calcium and phosphorous in the body, the matrix will mineralize. This process of remodeling is most obvious in its accelerated form when there is a fracture and the body quickly moves to repair the damage. On a more subtle scale, the body is constantly replacing old bone and repairing microfractures, the damage caused by daily activity. Repair of this damage helps keep the bones strong by preventing structural weaknesses from accumulating.

In children, bones grow because there is a very high level of both formation and resorption, but there is more formation and so a net gain in bone mass and density. Genetics heavily influences this process but bone also responds to environmental factors such as adequate intake of vitamins and minerals and activity levels. Stress placed on bones by physical activity stimulates growth that enables the bone to adapt to the rigors it is routinely exposed to. Even once physical growth is complete, bone tissue continues to be removed and replaced. This constant turnover sustains bone health but when levels of resorption and deposition become unbalanced certain disease states arise. Researchers estimate that peak bone density is achieved in young adulthood, with women reaching their peak bone density in their early to mid-20's and men, about a decade later (49). This time of peak density likely also represents peak strength for the bones. After this, there tends to be a gradual decline with age although the decline is neither inevitable nor consistent from person to person.

At any given time, bone is in one of three states: resorbing, forming, or quiescent (table 1). The vast majority of both cortical and cancellous bone is in the resting state at

any given time. In resting bone, the surface is covered by bone-lining cells. Despite the fact that most bone is in a resting state, there is a great deal of activity going on at the cellular level all the time and most of this activity occurs at the periosteal and endosteal surfaces. In general, remodeling increases the outside of bone diameter by adding to the periosteal side, while also increasing the diameter of the marrow cavity by eroding bone on the endosteal side. The level and distribution of remodeling activity depends on age, type of bone, disease state, mechanical usage, blood supply and hormonal influences. In healthy adults with a balance of resorption and formation, the daily release of calcium into the bloodstream from resorption is approximately 5 mmol of calcium. This is matched by deposition of an equal amount of calcium as part of the mineralization process that accompanies bone formation (36).

Table 2-1. Bone Metabolic States. Percentage of Human Cortical and Cancellous bone in the different metabolic phases (5)

BONE STATE	Cortical	Cancellous
Quiescent	97 %	93 %
Resorption	0.6 %	1.2 %
Deposition	3.0 %	6.0 %

The process of bone remodeling continues throughout life: resorption breaks down bone, osteoid fills the cavity and eventually becomes mineralized; woven bone is replaced by lamellar bone, old lamellar bone is replaced by new. In cortical bone, old osteons are resorbed and replaced; in cancellous bone, parts of the trabecular are resorbed and replaced (5). A major difference with cancellous bone, though, is that there is no way to replace rods that become broken.

Theories of Bone Remodeling

There has been a great deal of research to determine the process and stimulus for bone remodeling. While the mechanisms are still incompletely understood there are a myriad of known factors, some internal some environmental, that affect both the rate and the process. At one time, researchers believed decreases in bone density represented age-related reduction in the responsiveness of bone cells to hormonal and dietary influences, perhaps coupled with decreased availability of precursor cells that give rise to the osteoblasts and osteoclasts. Now there is a better understanding that the situation is more complex and open to change (27). Bone responds to external stresses such as exercise or the unloading that may occur with infirmity. In this respect it is similar to muscle tissue in that, within physiological limits, it adapts based on the load placed on it.

In 1892, Julius Wolff hypothesized that external stress influences bone architecture (32). Nearly 70 years later, Harold Frost postulated that it was not just mechanical strain that drove adaptation, but a “minimum effective strain” that influenced bone structure. In the 1970s, others added to the idea by suggesting it is dynamic rather than static stress that counts and that loading frequency also plays a role (82). More recent research attempts to determine what is happening at the cellular level to influence bone remodeling and determine its form and extent.

Much has changed since Julius Wolff first theorized about external influences on bone remodeling. Bone responds to external stimuli and there is clear evidence that mechanical loading positively influences bone density. We now know that dynamic loading is much more important for bone changes than is static loading. It also appears that the increase in loading need only occur for a short time to spur adaptive changes (82). If bone is not subject to sufficient external stimulation remodeling will shift activity

in favor of resorption. There is ample opportunity for the balance in bone metabolism to shift toward more resorption than deposition, however, since the osteoclast is able to dissolve in one day what it takes the osteoblast more than 10 days to fill in. This is why the gravity-free environment of space or extended bed rest (with no need to support the body's weight) results in rapid increases in bone resorption and bone thinning.

Mechanotransduction Theory

Mechanotransduction theory is the attempt to explain how the mechanical stimulus of muscle contraction pulling on bone translates into chemical action at the cellular level to change bone composition. This complex process, many believe, involves simultaneous mechanical, bioelectric, and biochemical processes, all occurring at the cellular level (20). Even though there are myriad influences on this process, it is possible to glean the role and importance of mechanical strain on the remodeling process.

According to this theory, normal daily activities maintain stress levels between 200 and 2000 microstrains (49). At this stress level, bone responds to bending forces that generate mechanical stretch. These stresses are sufficient to maintain bone integrity with no net changes in resorption or accretion.

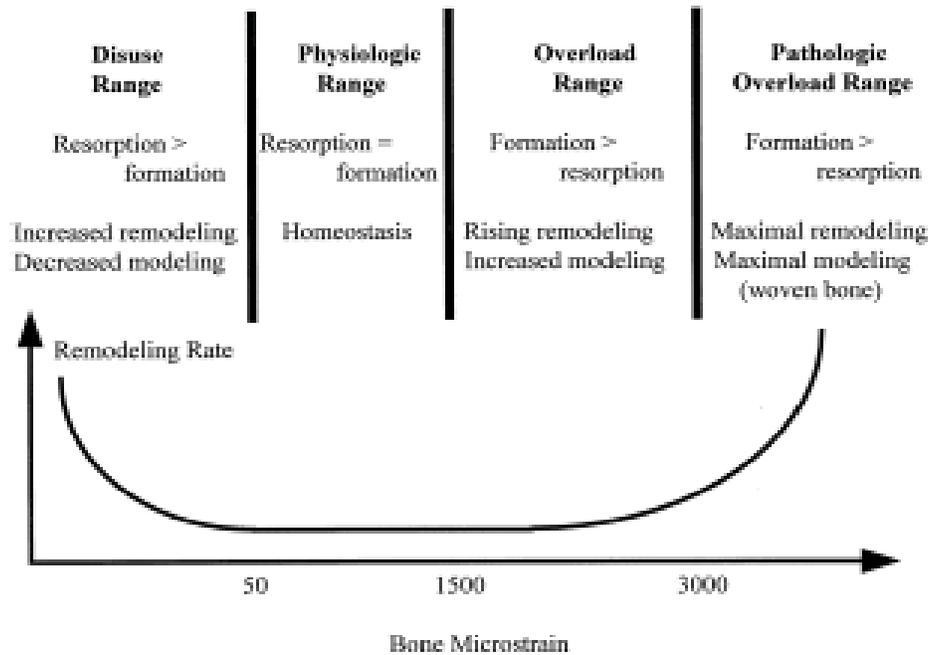


Figure 2-13 Relation Between Microstrain and Bone Remodeling Rate (49)

Many studies using animal models have tried to determine the minimal level of strain required to stimulate additional bone formation and researchers suggest 2500 microstrains as the level necessary to spur new growth (28). When the level of sustained strain exceeds the range of normal values, biological processes increase the amount of bone tissue deposited to meet the new “norm” (5). If the mechanical stimulus falls below the normal range of values, remodeling efforts remove bone (49). As Figure 2-13 shows, strain levels above and below the remodeling threshold will induce remodeling.

When exposed to strain, the bone experiences minute deformation, compresses, and moves the fluids in the microscopic cavities of the bone. This causes a change in the volume and pressure gradient of the interstitial fluid as it moves through the porous cavities of bone. Harold Frost and others believe (1998) that the mechanical results of muscle action included an increased flow of nutrients and movement of waste products from the underlying cells (2). This occurs through the canaliculi of the bone, which is

bathed in extracellular fluid and macromolecular complexes that have a composition slightly different from the mineralized matrix. Osteocytes detect these fluid shifts and then signal the change to bone lining and other cells(2).

Although the bone matrix surrounds the osteocytes, these cells are not isolated. Each osteocyte has up to 80 projections that reach into other canaliculi and through gap junctions in the bone. These processes are approximately 15 mm long and form a three-dimensional lattice connecting the osteocyte with the processes of up to 12 neighboring bone cells (20). The bone lining cells, osteoblasts, and osteocytes connect via this lattice of cell projections and form a syncytium. Current research suggests that the osteocyte syncytium permits signaling between neighboring osteocytes. The cytoplasmic projections provide the osteocyte with information about its environment and allow cell signaling between osteocytes and between osteocytes and other cell types. Gap junctions also permit ions to pass between two cells without having to pass into the extracellular space and connect the superficial osteocytes with bone lining cells and osteoblasts on the periosteal and endosteal bone surfaces (20). Researchers point out that chemical signals would be too slow and so argue this represents electrical cell-to-cell communication (20). Osteoblasts also take part in cell signaling through two osteoblast-mediated proteins: RANK ligand, which stimulates osteoclast production by binding to its receptor RANK on osteoclast precursors, and osteoprotegrin, which binds RANK-ligand to inhibit its interaction with RANK. When signals increase bone remodeling, RANK-ligand production increases. It then binds to RANK, which initiates osteoclast proliferation (46). When remodeling decreases, RANK-ligand production lessens. Osteoprotegrin is

then secreted to compete with RANK as a “decoy” receptor and osteoclast production declines (46).

Although researchers used mechanotransduction theory to explain increases in bone formation, it does not adequately explain the rapid loss of bone density under conditions of unloading. More recent research helps fill this information gap by proposing that bone lining cells are part of the signaling mechanism and that they stimulate a certain level of bone remodeling activity unless presented with inhibitory signals from the osteocytes (49). Under this theory, bone-lining cells contain sensory cells that monitor mechanical strain and compare it to a relatively narrow range of norms. When the level of sustained strain exceeds the range of normal values, microdamage occurs which stimulates the formation of additional bone to meet the new “norm.” If mechanical stimulus falls below the normal range of values osteocytes begin to experience increased apoptosis which disrupts the inhibitory signals to the bone lining cells and causes increased resorption (49). Nobel and his colleagues (2000) believe that apoptosis of osteocytes releases substances that serve as a homing signal for osteoclasts and thus expedites bone resorption where it is needed (58).

This idea helps explain the effects of aging as well since aging brings with it a significant decrease in the number of viable osteocytes. While researchers have found that typical bone contains less than 1% dead osteocytes in young children, that figure rises to 75% by age 80 (84). Even those osteocytes that survive may become less effective since their estrogen receptors are no longer being acted upon by estrogen (83). Increased apoptosis of osteocytes, according to Martin et al., decreases inhibitory signals to the

bone lining cells which, in turn, increases bone remodeling (49). Figure 2-14 illustrates communications between osteocytes and bone lining cells.

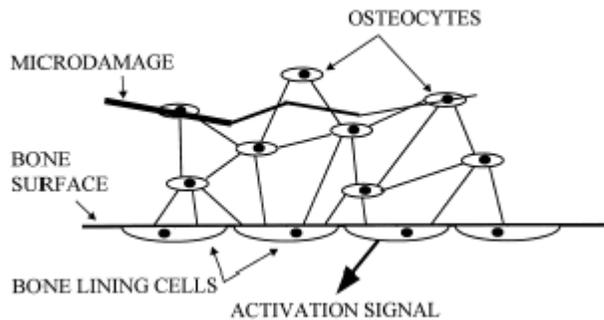


Figure 2-14. Osteocyte Signaling Pathway (49)

Basic Multicellular Units (BMUs) are the chief instrument for turning bone over, removing mechanically unneeded bone, and repairing microdamage by laying down new bone. BMUs consist of osteoclasts and osteoblasts that congregate on a specific area of the bone where they have been drawn by the signaling activity of osteocytes (27). The BMU goes through 6 separate and distinct phases: resting, activation, resorption, coupling, formation, and mineralization.

Once bone ends its resting phase and turnover processes are activated, bone resorption occurs first and is carried out by osteoclasts; refilling of the resorption cavity with osteoid secreted by osteoblasts follows this. In the final phase, if there is enough calcium available, the new bone is mineralized. The entire sequence of resorption and formation at a given remodeling site takes place over a period of several months; this process is being carried out at many sites simultaneously, though each site may be at a different phase of the process (33). Researchers do not fully understand the activation stimuli for bone remodeling, but believe they relate to structural and biomechanical requirements. Since bone lining cells contain PTH and 1-25-(OH)₂ vitamin D₃ receptors

and osteoclasts do not, it has been suggested that bone lining cells, when stimulated, start the resorption process by digesting the endosteal membrane of the bone. This exposes mineralized bone surface which acts as chemoattractant for osteoclast precursor cells (5, 49, 50).

The resorption process begins when the multinucleated osteoclast arrives at the remodeling site. The osteoclast, with its ruffled border, attaches via a clear zone to the resorption site and it is here that the osteoclast and the underlying bone form a distinctly acidic microenvironment. When, the osteoclast secretes acids into this microenvironment, the matrix collagen breaks down and forms concave pits called resorption pits or Howship's lacunae. The resorption pits have an average erosion depth of 60 micrometers in trabecular bone and about 100 micrometers in cortical bone. The osteoclasts can erode up to tens of micrometers per day (49). The whole process of resorption takes 1-3 weeks and culminates with the release of calcium from the dissolved bone. The end of resorption is marked by osteoclasts migrating from the endosteal surface to nearby marrow spaces, where they hibernate or die, having lived about 7 weeks (5, 49, 50). Following this, osteoblasts migrate into the area.

Coupling is the term used for the process of osteoblasts migrating to sites of resorption. Coupling insures osteoblasts are attracted almost exclusively to sites of resorption (36). The coupling mechanism appears to be locally regulated and triggered automatically. It may be that there is a release of osteoblast mitogens from the previously resorbed bone that attracts osteoblasts (5). There is a 1- or 2- week time period between the end of resorption and the beginning of new bone formation (5). This time, called the coupling period, is characterized by a lack of osteoclasts in the resorption pits. These

pits, however, do contain mononuclear cells of unknown origin and function. They may be osteocytes, preosteoblasts, or even fractured parts of the multinucleated osteoclasts.

Many scientists believe osteoblasts are stimulated to mature by signals from the compounds released by the bone itself when it breaks down. Some believe these signals occur when the calcium released by resorption activates calcium receptors on osteoblasts. This, theoretically, would insure bone resorption does not become uncontrolled, since the more resorption there is the more osteoblasts that would be stimulated to form new bone (33). Several substances stimulate osteoblast formation and activity, including transforming growth factor- alpha and -beta (TGF-a and TGF-B), bone derived growth factor (BDGF), insulin-like growth factor (IGF-1), skeletal growth factor (SGF or IGF-2) interleukin-1 (IL-1), macrophage derived growth factor (MDGF), transforming growth factor, alpha (TGF-a) prostaglandin E₂(PGE₂) and systemic growth factors like epidermal growth factor (EGF), platelet derived growth factor (PDGF) and tumor necrosis factor – alpha and beta (TNF). Additionally, hormones such as estrogen, growth hormone, insulin, parathyroid hormone, testosterone and agents like fluoride and aluminum that directly or indirectly stimulate bone formation to varying degrees (5, 49, 50).

Columnar preosteoblasts appear about 9 days before matrix synthesis begins (5). Once the preosteoblast cells mature, they secrete osteoid. This occurs over about a 15-day period and is the formation stage. Once the new osteoid matrix reaches about 20 micrometers in depth, mineralization begins. Mineralization phase takes place at the junction of the osteoid and the osteoblasts, a region known as the mineralization front. Mineralization lags behind osteoid formation, by about 10 -15 days and proceeds based

on the availability of calcium and phosphate, the primary ions involved in forming hydroxyapatite (5). While the initial mineralization takes place rather quickly, it may take up to several months for all the osteoid to fully mineralize explaining why the formation of new mineralized bone is not immediately detectable. Researchers estimate it takes at least three months to achieve a level of mineralization sufficient to measure with techniques such as a DXA scan.

If bone remodeling balance shifts in favor of resorption as it often does with aging, bone weakens and fracture risk increases. Bone density has been repeatedly shown to correlate with increased bone fracture rates (35). The change in amounts of bone resorbed and deposited does not have to shift much to make a meaningful difference. Researchers have estimated that in osteoporosis bone releases an average of 6 mmol of calcium daily while 5 mmol is replaced (36). Although this seems like only a slight imbalance, it equates to an annual bone loss of 1% per year (36).

Bone Adaptation at the Cellular Level

Research efforts concerning the underlying mechanisms of bone adaptation to external stress center on activity at the cellular level. Although there is still much work needed to illuminate the process, certain themes are emerging from current research. As a result of mechanical loading, there is a rapid increase in nitric oxide (NO) prostaglandins I₂ and E₂ (PGI₂ and PGE₂) (37, 51). In addition to mechanical loading, there are also several endogenous influences on levels of bone metabolism. PTH, 1,25 dihydroxyvitamin D₃, PGE₂, PGI₂, interleukin-1 (IL-1), tumor necrosis factor (TNF)-alpha and TNF-beta, to name a few, stimulate bone resorption (34, 40). These activate osteoclasts through osteoblast mediation (since it is the osteoblasts that have receptors for these) and osteoclast differentiation (34). Agents such as endotoxins, vitamin A,

thyroxin, and acidosis stimulate osteoclast resorption through incompletely understood pathways (31). Inhibitors of bone resorption include calcitonin, and calcitonin-related peptides that act directly on osteoclasts and prevent their activation.

Strain increases the production of cytokines, such as interleukin-1beta (IL-1B), Tumor Necrosis Factor-alpha (TNF-a), and Interferon-gamma (IFN-gamma) which activate the Nitric Oxide Synthase (NOS) pathway. This increases the production of inducible NOS (iNOS) and, in turn, NO. Endocrine NOS (eNOS) is less dependent on cytokine production but also contributes to the rapid production of NO from L-Arginine. When exposed to strain, both osteoblasts and osteocytes become more responsive to NOS and produce NO. This is not surprising since these cell types share the same lineage.

Interleukin-1 is a cytokine that regulates bone resorption and formation. It is the most potent endogenous stimulus for resorption known (34). Its role in bone formation is likely inhibitory since it also increases prostaglandin synthesis and prostaglandins are powerful agents of resorption. Tumor Necrosis Factor, which is stimulated by IL-1, is also a potent stimulator of resorption and inhibits the synthesis of bone collagen when it is present in large amounts. In smaller amounts, however, it increases collagen synthesis by osteoblasts (34).

Researchers are just beginning to understand the role of NOS and NO in bone remodeling. We know that eNOS is constitutive and is regulated post-translationally while iNOS is regulated at the transcription level. Neural NOS (nNOS) acts via the binding of calmodulin to enzymes when the levels of intracellular free calcium increases as a result of external strains on the bones, while iNOS appears to operate independent of calcium levels (39).

Nitric Oxide is a powerful signal transduction effector molecule. It is produced in macrophages, neutrophils, bone marrow and by osteoblasts and osteoclasts when they are exposed to the cytokines IL-1, TNF, and IFN (18). Nitric oxide has dose-dependent effects on bone. In large amounts it inhibits bone growth, while in smaller amounts it inhibits bone resorption (39). At first glance it may seem contradictory that many of the substances secreted in response to mechanical strain stimulate bone resorption when the adaptive response is bone formation. It is not contradictory, though, since resorption always precedes formation and has to occur first to achieve the desired result.

Nitric oxide is released rapidly following increased stress levels and this is thought necessary for the subsequent release of prostaglandins (20). The formation of NO causes an increase in Arachadonic Acid. NO also forms a complex with the heme of cyclooxygenase (COX). Since Arachadonic Acid and COX are required to form prostaglandins, it is not surprising that the production of prostaglandins also increases. Prostaglandin G/H synthase converts Arachadonic acid to PGG₂ in a cyclooxygenase reaction. Through another series of reactions, PGG₂ is converted to PGE₂ and PGI₂ (63). Researchers have shown the connection of NO with the production of prostaglandins by blocking NO production and measuring the resulting effect on prostaglandins. One study documented a 30% decline (51). It also appears that NO influences the basal metabolism of the osteoblasts (24, 44, 51).

Many important regulators of bone metabolism stimulate prostaglandin production. Cyclic AMP increases prostaglandin production while an increase in prostaglandins stimulates cAMP production via a positive feedback system (63). Fluid stress also increases prostaglandin production. Fluid stress increases mRNA levels suggesting that

bone cells respond to increased stress by expressing a variety of genes. For example, Type I collagen has the amino acid structure GLY---PRO---PRO and PGE₂ stimulates production of the amino acid proline. The mitogenic effect of PGE₂ appears to be greatest when there is brief exposure. Prolonged exposure decreases the adaptive response (63).

The Role of Estrogen

The purpose of this study is to determine the ability of resistance exercise to increase BMD in postmenopausal women who are not receiving hormone replacement therapy. It is important, then, to understand the complex interaction between estrogen and bone. Researchers have long understood that estrogen is bone protective and that the loss of estrogen accompanying either surgical or natural menopause leads to increased bone loss in women. A marked increase in bone resorption characterizes the postmenopausal period. What is less understood, is why estrogen withdrawal has such a profound effect. Researchers once believed estrogen acted almost exclusively by suppressing osteoclasts, but now it is clear that the situation is much more complex. Three of the major types of bone cells osteoblasts, osteoclasts, and osteocytes all have estrogen receptors so withdrawal of estrogen has wider effects than just increasing osteoclast activity.

Prior to menopause, estrogen increases apoptosis in osteoclasts and this helps keep bone resorption down (81). More than this, though, estrogen inhibits the differentiation and recruitment of osteoclasts (84). There is also evidence that, in the presence of estrogen, osteocytes generate osteoprotegerin, a member of the tumor necrosis factor (TNF) receptor superfamily, which is known to inhibit osteoclast formation and differentiation (41, 58).

With a loss of estrogen at menopause, there is an upsurge of both osteoclastogenesis and osteoblastogenesis (68, 88). Osteoclast apoptosis declines, resulting in a 10-15% increase in the number of viable osteoclasts (68). With increased osteoclasts comes increased resorption. The breakdown of bone increases calcium levels in the blood. This, in turn, increases renal calcium filtration. Exacerbating this, estrogen receptors in the kidney respond to the lack of estrogen by decreasing calcium reabsorption by the kidney. These two factors help explain why estrogen depleted women have increased calcium excretion via the urine (17, 46).

Increased calcium levels in the blood also leads to decreased PTH secretion. This reduces renal synthesis of 1,25 dihydroxy vitamin D which, in turn, reduces calcium absorption through the small intestines (46). Estrogen is also known to down regulate gene expression of IL-6, a powerful stimulator of bone resorption and osteoclast recruitment (83). It is no surprise, then that there is a marked increase in IL-6 production following menopause (72, 81).

The effect of estrogen on osteoblasts is less clear-cut, and researchers are still not sure whether estrogen directly effects osteoblasts or whether increased osteoblast activity is in response to increased osteoclast activity (83). Whatever the effect, there is both increased resorption and increased accretion in the postmenopausal period the net shift is decidedly in favor of resorption. While osteoclasts become more numerous and active, osteoblasts become less efficient, although it is unclear whether it is the osteoblasts themselves that are affected or if the inefficiency lies in the coupling process, which draws both osteoclasts and osteoblasts to a resorption site (83).

The loss of estrogen also increases apoptosis in osteocytes (81). Osteocyte numbers decline by 8.6-13% (83). Some researchers believe that increased osteocyte apoptosis is due to the loss of estrogen's antioxidant effects or that somehow a lack of estrogen compromises the osteocyte's viability (58). Martin et al. (49) and Noble (58) suggest that at menopause the lack of estrogen triggers osteocytes, which have estrogen receptors, to decrease inhibitory signals to the bone lining cells. The overall result is that the loss of inhibitory signals to the bone lining cells results in increased bone turnover, with metabolism favoring resorption versus apposition.

During the first 5-7 years (some researchers say 10 years) after menopause begins there is an accelerated rate of bone loss, on the order of 3% per year. After this period of accelerated loss, the rate diminishes to about 1% per year (17, 61). In a large-scale study, researchers also found that in healthy older men and women that there is a natural increase in osteoprotegerin, a substance that inhibits bone loss, after the age of 60 in women and the age of 70 in men (41). At this point, no one knows what causes this increase, but it may explain why the accelerated loss of bone in women right after menopause seems to moderate after 5-10 years.

Inducing Bone Formation Via Exercise

Resistance training as a means of increasing bone density has received a lot of attention but the results of randomized studies are contradictory. Lohman et al.(43) studied the effects of 18-months of resistance training on regional and total BMD, lean body mass and strength in premenopausal women. They found that most BMD gains occurred within 5 months of beginning the exercise program. While strength increased significantly, BMD increased only regionally, not globally. Lumbar spine density increased by an average of 2.0% after 5 months but declined to 1.4% above baseline by

the end of the study. In the hip, only trochanter density showed a significant change (1.8%) at 12 months and increased to 2.0% at two years. The authors concluded that their data represented a redistribution of BMD (43).

Friedlander et al. (25), who conducted a study of the effects of a combined aerobic and strength training program in young women over a two-year period, found that exercise intervention did improve bone regionally. Her group achieved increases of 2.5% in the lumbar vertebra, 2.4% at the femoral neck and 2.3% in the femoral trochanter. Interestingly, while one year of intervention showed promising results, they only achieved statistical significance at the 0.05 level during the second year of the study. Another study of premenopausal women, conducted at the Mayo Clinic tested the effectiveness of non-strenuous spinal and femur muscle strengthening program on BMD showed no significant gains over the three year length of the study (77). While the exercise group had a slight increase in bone density and the control group had a slight decrease in density the effect was not significant at the three-year point. Reasons for a lack of progress are unclear, but this protocol only called for exercise once per week and this may not have been a sufficient stimulus to elicit changes in such a young subject population. Additionally compliance with the training program decreased significantly over time. By the third year of the study, only 60% of the women continued with their exercise programs.

There is also a significant body of research on postmenopausal women and the effects of exercise on their bone density. Nelson et al. (57) assessed the effects of a twice weekly, high intensity resistance training program on postmenopausal women not taking hormone replacement therapy. They found that femoral neck BMD increased by 0.9%

and lumbar spine BMD increased by 1.0% after one year. The exercise protocol protected total body BMD in exercise subjects while total body BMD in control subjects declined by 1.2%.

Maddalozzo and Snow (45), working out of the Bone Research Lab at Oregon State University, looked at differences in how a 24-week moderate or high intensity free weight exercise program would influence lumbar and hip bone density in older men and women. In this study, men improved their lumbar spine bone density under the high intensity exercise program only while women showed no improvement in either the hip or the lumbar spine under either exercise program. This was despite a 37.62% strength gain. They speculated that low intensity exercise was not intense enough to elicit adaptations in women with low estrogen levels (45).

Bassey and Rothwell (1) tested the effects of a protocol calling for vertical jumping exercises on BMD in both pre- and postmenopausal women. The protocol, which called for 50 vertical jumps to a height of 8.5 centimeters six days/week resulted in significant (2.8% gains in femoral BMD for premenopausal women but achieved no significant result for postmenopausal women even after 18 months.

Kerr (38) looked at the effect of a one-year progressive resistance training program (designed either for strength or endurance) on the hips and forearm of older women. Both groups showed significant increases in strength, but only the strength group showed significant improvement in BMD. The increases in BMD were site specific, with the researchers attributing increases in trochanter BMD to the leg press exercise. These results suggest that in older women programs that use high-load, low repetition are the most successful (38).

Specific exercise machines may prove particularly effective in inducing new bone formation. Pollock et al. (64), using the same MedX resistance training equipment described in this research proposal, found that 6 months of lumbar extension exercise increased BMD of the lumbar vertebra (L2-L3) by 14% in elderly subjects (n=50), but treadmill walking or stair climbing exercise had no effect on BMD. Vincent and Braith. also using the same MedX resistance training equipment described in this research proposal, found that 6 months of leg press exercise increased BMD of the femur neck by 2% in adults aged 60-83 years (n=22), but the age-matched control group (n=16) that did not perform leg press exercise experienced mean losses of 1.5% in femur neck BMD (86).

CHAPTER 3 METHODS

Introduction

This study was designed to determine the effects of a progressive resistance training program on the bone density of postmenopausal women not currently taking hormone replacement therapy. Recently raised concerns over the long-term use of hormone replacement therapy (HRT) by postmenopausal women make this study timely since many women are now reluctant to take HRT and are looking for alternative means of protecting their bones. Resistance exercise is recognized as osteogenic but the exercise type and dose most effective for estrogen-depleted postmenopausal women remains unclear. This prospective study will attempt to answer these questions.

To assess the effects of a progressive resistance training program, the postmenopausal women were assigned to one of three exercise protocols. One group exercised using nine pieces of exercise equipment typically found in commercial gyms. A second group used these same nine machines plus the MedX leg press and medical lumbar extension machine. The resistance training regimen for group 2 represented our attempt to determine if these two machines had any additive osteogenic effect for the hip and lumbar spine respectively. A third group, represented an endurance exercise control group and subjects walked on a treadmill.

We were also interested in determining if the anti-osteoporotic drugs currently available (Fosamax, Actonel, Evista) provide any additive value when combined with exercise. Therefore, at study entry women were identified as either taking or not taking

anti-osteoporotic drugs. Those women currently taking any of the anti-osteoporotic drugs were assigned to a single resistance exercise group so that the additive effects of the drugs, if any, could be quantified. Thus, there were four groups in the study (Figure 1).

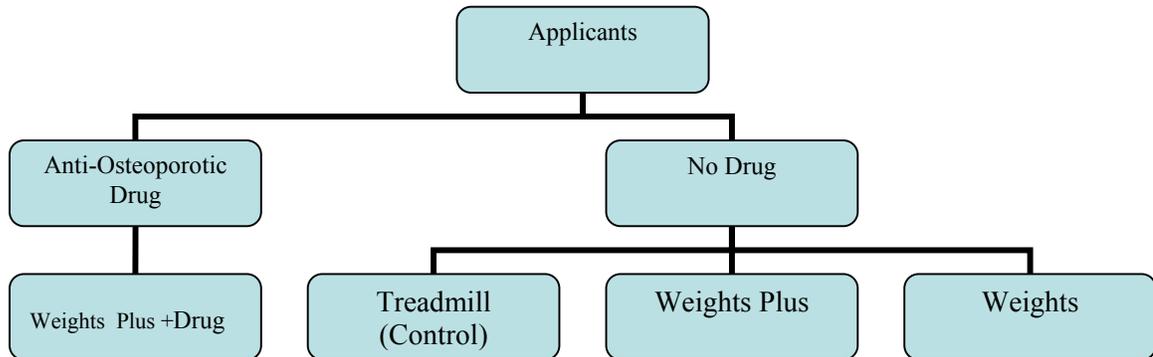


Figure 3-1. Study Group Assignments

It is necessary to compile results for those on anti-osteoporotic drugs separately from those not on the drugs since these drugs would be expected to result in increased bone density even in the absence of a regular exercise program. Applicants using an anti-osteoporotic were all assigned to a training regimen that included 11 exercise weight machines. We did not recruit enough subjects using anti-osteoporotic drugs to be able to randomize them to all of the exercise protocols. Therefore, they were all be assigned to one group. Those subjects using no anti-osteoporotic drug were randomized to one of three exercise groups: treadmill walking, 9 exercise machines, or 11 exercise machines.

Subjects

Subjects were recruited using fliers and advertisements placed in the local newspaper. To qualify subjects had to:

- Be Between 50 and 75 years of age

- Be Sedentary
- Have been postmenopausal for at least one year
- Still have their ovaries or had them removed after age 50
- Not have been on hormone replacement therapy (HRT) for at least six months
- Be non-smokers
- Have blood pressure less than or equal to 140/85
- Not have had a bone fracture within the past year
- Have no health problems that would contraindicate participation in an exercise program
- Be taking no medications that influence bone metabolism (except antiresorptive drugs)
- Not have bone density more than 3.0 standard deviations below that of healthy young adult females at peak bone density (T Score > -3.0)
- Not be currently engaged in a regular exercise program.

After acceptance to the study women were excluded if they:

- Failed to regularly attend exercise sessions
- Became unable to continue their assigned exercise program
- Had a diet analysis that indicated they are not getting a minimum level of 1000mg of calcium per day and 1.1g protein per kg body weight
- Sustained injuries that make participation unfeasible or unsafe
- Changed their medication status in ways that would affect the study (such as taking statin class drugs, starting or stopping anti-osteoporotic drugs, etc)

Acceptance into the Study

Women answering the study advertisements who appeared to be qualified for the study were invited to come to the University of Florida to fill out a health survey, have their blood pressure measured and tour the exercise facility. All subjects filled out a health survey to determine their general health and detect any condition or medication

that might adversely affect the study results (Appendix A). Once the health surveys were completed, they were sent to Dr. Michael Fulton, Medical Director for the study, who approved or disapproved their enrollment into the study. Subjects were advised of the benefits and risks of the study and signed an Institutional Review Board (IRB)-approved informed consent (Appendix B).

Medically qualified candidates received a Dual Energy Xray Absorptiometry (DXA) scan (total body, lateral lumbar and non-dominant hip) prior to final acceptance to ensure they were not more than 3.0 standard deviations below normal for healthy adult females at peak bone density.

Group Assignments

Once approved for the study, subjects not taking anti-osteoporotic drugs were randomly assigned to one of three exercise groups: treadmill-walking, using nine resistance exercise machines, or using eleven resistance exercise machines. Group assignment was determined by block assignment according to whether they were currently taking an anti-osteoporotic drug and then by order of acceptance into the study. Participants were not allowed to select their own group assignment nor were they able to switch groups. Other researchers studying the effects of exercise on bone formation have noted that results can be affected if subjects participate in their own group selection (89). Wolff found that results were more favorable under these circumstances suggesting that subjects with a preference for a particular type of exercise may achieve better results when a randomized design is not used, but these results are not representative.

Equipment Familiarization and Strength Testing

Participants reported to the Center for Exercise Science at the University of Florida and performed tests designed to measure skeletal muscle strength at study entry and

following 4.5 and 9 months of exercise training. All subjects were provided with familiarization training on the treadmill and resistance exercise equipment. After the subjects were familiarized with the exercise machines, a one-repetition maximum strength test (1-RM) was performed on six different machines: Leg Press, Chest Press, Torso Arm, Leg Extension, Overhead Press, and Seated Row.

A one-repetition maximum (1-RM) is defined as the greatest amount of weight that can be lifted one time through the entire range of motion using proper form. Subjects first were asked to walk for five minutes on a treadmill as a generalized warm-up. Then, they were instructed on the proper form for each exercise and were asked to practice and do a warm-up on the machine using 6-8 repetitions at approximately 60%-80% of the expected maximum weight. Following the warm-up and a one-minute rest period the weight was increased and the subject attempted to lift the weight. If they were successful, the weight was again increased and they attempted another lift following a one-minute rest period. This procedure continued until they could either no longer lift the weight or could not lift it while maintaining proper form. Every attempt was made to get to the maximum lift within 5 attempts to avoid muscle fatigue.

Isometric strength of the lumbar extensor muscles was measured using the MedX lumbar extension machine. During this evaluation, the patient first complete done set of warm-up exercises for the lumbar extensor muscles with a light weight. Subjects exercised from flexion to extension through their full range of motion (ROM). After a brief rest, the subject then completed an isometric test of lumbar muscle strength. Six testing positions (0, 12, 24, 36, 48, and 60) degrees of lumbar flexion) were measured. A maximal isometric contraction is generated at each of these angles. Subjects were

instructed to extend their back by building up tension over a two to three second period. Following each isometric contraction there was a ten second rest period while the next position was set. In this manner, a torque (strength) curve was generated throughout the ROM for each subject.

Anthropometric Measures and Bone Mineral Density Scan

Individuals accepted into the study reported to the Center for Exercise Science at the University of Florida where they had their height and weight measured. They then had a measurement of whole body and regional bone mineral density (BMD) using a dual energy X-ray Absorptiometry (DXA)(Lunar Radiation Corp., Madison, WI). DXA is a non-invasive technique that uses low-power X-ray energy to measure total body and regional BMD. With the subject supine or on their side, the X-ray scanner performs a series of transverse scans at 1 cm intervals. Bone mineral density (g/cm²) of the total body, femur neck, lumbar spine, and distal radius was measured. This procedure was performed by a licensed X-ray technician and took approximately 40 minutes. The purpose of this procedure was to establish baseline BMD values before the exercise intervention began. This test was repeated after 4.5 and 9 months of exercise training to determine the effects of the exercise protocols on bone density. Participants were also offered the opportunity for annual bone scans for a period of five years after the exercise program ended. This will document the long-term effects of the exercise intervention. Subjects who complete the study will be notified by mail 60 days prior to their completion anniversary. They will be provided with the phone number for the DXA lab and instructed to call to make an appointment so that each measurement is taken within two weeks of the anniversary of their T3 scan. We will follow up with a phone call if they fail to contact us within 45 days of their completion anniversary.

Blood Testing

Blood was drawn by venipuncture from an antecubital vein using a 21-gauge needle at baseline and at 18, and 36 weeks into the study. On the day of the blood draw, two 10 ml red top serum tubes and two 10 ml purple EDTA plasma tubes were drawn before exercise and one red top and one EDTA purple top plasma tube were drawn after the normal exercise protocol. Although we were not able to draw blood from all of the subjects at a specific time of the day, consistency was maintained for each subject to minimize the effects of diurnal changes. Subjects were not required to fast prior to blood collection. Once the blood was collected the EDTA plasma tubes were refrigerated for 15 minutes, while the serum tubes were kept at room temperature for 15 minutes. The plasma and serum tubes were then centrifuged at 5000 RPM for 15 minutes. The serum and plasma were then divided into 1 ml aliquots and stored at -80 degrees centigrade. At the end of the study, all samples were for each biochemical assay were processed at the same time. Blood samples were used to determine serum levels of osteocalcin and pyridinoline crosslinks which are biochemical markers of bone turnover. Serum markers of bone formation and resorption provide a basis for explaining alterations in bone mineral density.

Osteocalcin

Seiffert-Klaus et al.(74) examined levels of various serum markers in pre- peri- and postmenopausal women, and found that pyridinoline levels begin to increase during perimenopause than remain relatively stable during menopause. Osteocalcin levels tended to increase both at perimenopause and then again as women enters menopause.

Serum osteocalcin testing was done using a Metra Osteocalcin EIA kit (Quidel Corporation, San Diego, CA). The Metra osteocalcin assay is a competitive

immunoassay. The assay uses osteocalcin-coated strips, a mouse anti-osteocalcin antibody and an anti-mouse IgG-alkaline phosphatase conjugate to detect and measure osteocalcin in serum. All biochemical assays were performed in Dr. Braith's laboratory.

Pyridinoline

Pyridinoline testing was done using a Metra Pyridinoline EIA Kit (Quidel Corporation, San Diego, CA) The serum pyridinoline assay is a competitive immunoassay in a microtiter plate form. Seiffert-Klaus et al. (74) conducted studies of pyridinoline levels in pre- peri- and postmenopausal women to determine age-related changes in this marker of bone resorption. She found that levels of pyridinoline rise somewhat during perimenopause but then climb sharply as women enter menopause.

Testing Protocol: Aerobic Training

The endurance training protocol (Treadmill group) walked twice weekly on a treadmill for 30-40 minutes at a speed and elevation that represented 65-80% of age-predicted heart rate reserve. Peak heart rate was estimated by subtracting subject age from 220. Subjects in the aerobic training group began the training program gradually, by walking for 20 minutes at 50% of their maximal Heart Rate Reserve (HRR). Walking duration increased by 5 minutes each week until the subjects were walking for 40 minutes. At week 8, treadmill speed and/or elevation was increased as necessary to keep subjects working at 65-80% of their HRR. Training intensity was gradually increased until subjects were walking at 70-80% of HRR. Treadmill speed was capped at approximately 3.5 mph, however, to avoid subjects having to jog or walk too fast. An exercise specialist monitored each participant during exercise-training sessions. Training sessions began with 5-10 minutes of light treadmill walking.

Testing Protocol: Resistance Training

There were two resistance training groups (Weights and Weights Plus/Weights Plus + Drug). Both groups will be oriented to the MedX variable resistance exercise machines.

Weights Plus. This group performed a regimen of variable resistance exercises, 2 days per week, on the following MedX equipment: Leg Extension, Leg Curl, Torso Arm, Seated Row, Chest Press, Overhead Press, Biceps Curl, Triceps Pushdown, and the Abdominal machine. This equipment is designed to work all major muscle groups. In addition, Group 2 also exercised on the MedX clinical lumbar extension and leg press machines (total 11 machines). The initial resistance for each subject on each machine was 50% of the participants 1-RM. Subjects completed one set of 8 to 12 repetitions performed to volitional fatigue per machine (20 repetitions on the lumbar extension machine). When 12 repetitions were achieved during two consecutive training sessions (20 on lumbar machine), the training weight was increased approximately 5% at the next training session. Progression was based on each individual's adaptation. An exercise specialist monitored each participant during all exercise-training sessions. Each testing session began with 5 minutes of light treadmill walking.

Weights. Group 3 subjects performed a regimen of variable resistance exercises, 2 days per week, on the same MedX equipment as Group 2 but they did not exercise on the MedX clinical lumbar extension or the MedX leg press machines (total 9 machines). The initial resistance for each subject on each machine was 50% of the participant's 1-RM. Subjects completed one set of 8 to 12 repetitions performed to volitional fatigue per machine. When 12 repetitions were achieved during two consecutive training sessions, the training weight was increased approximately 5% at the next training session.

Progression was based on each individual's adaptation. An exercise specialist monitored each participant during all exercise-training sessions. Each training session began with 5 minutes of light treadmill walking.

Power Analysis

Analysis was performed to estimate the statistical power related to testing the following hypothesis in 60 postmenopausal women: 36 weeks of supervised resistance exercise training supplemented with MedX lumbar extension and leg press machines will result in greater gains in bone mineral density in the hip and lower lumbar region and total body than resistance training without the lumbar extension and leg press exercises or an aerobic exercise program. The results of the power analysis indicated that 9 participants per cell would result in a power of 88% at an alpha level of 0.05. Based on this, a total of 60 participants will be recruited (fifteen per cell) for this study.

Statistical Analysis

Descriptive characteristics was compared between groups using analysis of variance (ANOVA). When a significant group by time interaction is observed, within-group comparisons between time points and between-group comparisons at each time point will be performed using ANOVA with contrast analysis for obtaining appropriate post-hoc custom hypothesis tests. All statistical analyses will be performed using the SPSS statistical program. An alpha level of $p \leq 0.05$ will be required for statistical significance.

CHAPTER 4 RESULTS

Measurement Design

A total of 46 subjects completed the study. Subjects recruited for the study were measured at study entry (T1), and after 4.5 months (T2) and 9 months (T3) of exercise intervention. Bone density scans, using central DXA, examined regional changes in levels of hydroxyapatite in g/cm^2 of the total body, lumbar spine, and non-dominant proximal femur (neck and trochanter). Body composition analysis was conducted using DXA. Body composition measurements included percent fat, Total Tissue (kg), Fat (kg) and Lean tissue (kg) and Bone Mineral Content (kg). Strength testing was also conducted at T1, T2 and T3. Lumbar extensor muscle strength was measured using a 6-angle isometric test with the MedX medical lumbar extension machine. 1-Repetition maximum (1-RM) testing was conducted using MedX Chest press, Torso Arm, Overhead Press, Seated Row, Leg Extension, and Leg Press equipment. Serum markers of bone resorption (pyridinoline crosslinks) and formation (osteocalcin) were also collected at T1, T2, and T3.

Subject Characteristics

Descriptive characteristics at study entry and study compliance rates were measured in subjects and the results are shown in Table 4-1. There were no statistically significant differences in descriptive characteristics between the subjects assigned to the four study groups at T1. There were also no statistically significant differences in

exercise training compliance rates between groups. In aggregate, compliance of subjects in all groups averaged 86.85% over the duration of the 9-month study.

Table 4-1. Subject Characteristics

Characteristics	Control (n=12)	Weights (n=12)	Weights Plus (n= 12)	Weights Plus + Drug (n=10)
Age (yrs)	59.9±5.9	57.3±4.5	57.8±6.5	57.7±3.7
Height (in.)	63.9±3.0	62.4±3.6	65.3±3.7	63.4±2.9
Weight (kg)	70.1±14.7	64.6±7.4	71.0±14.2	63.7±5.6
Total Body BMD (g/cm ²)*	1.11±0.09	1.10±.04	1.12±.10	1.05±.05
Compliance (%)	82.67±7.8	87.8±7.0	90.61±6.5	87.28±5.3

Values are expressed as mean ± standard deviation. * Hydroxyapatite

Bone Density

Bone density measurements were performed on 46 subjects. There were no statistically significant differences in regional BMD of the study groups at the start of the study.

BMD was serially measured in four anatomic regions:

- Total Body
- Femur Neck
- Femur Trochanter
- Lumbar Spine.

Total Body Bone Mineral Density

The means and standard deviations for total body bone mineral density are contained in Table 4-2. Absolute changes in BMD are shown in Figure 4-1. Relative changes in Total body BMD are shown in Figure 4-2.

The only significant change in Total body BMD between T1 and T2 occurred in the Weights Plus + Drug group (1.050 to 1.036, (p<0.01). The only significant change in

Total Body BMD between T1 ad T3 also occurred in the Weights Plus + Drug group
(1.05 to 1.042, [<0.05])

Table 4-2. Bone Mineral Density Measurements.

Treadmill	T1	T2	T3	% Change T1-T2	% Change T1-T3
Total Body	1.112 \pm 0.8	1.111 \pm 0.09	1.108 \pm 0.82	-0.089	-0.360
Femur Neck	0.845 \pm 0.12	0.840 \pm 0.11	0.828 \pm 0.12	-0.592	-2.01
Trochanter	0.772 \pm 0.12	0.769 \pm 0.12	0.767 \pm 0.13	-0.389	-0.648
Lumbar Sp	0.662 \pm 0.10	0.635 \pm 0.13	0.610 \pm 0.11	-4.079	-7.85
Weights					
Total Body	1.102 \pm 0.04	1.098 \pm 0.04	1.101 \pm 0.05	-0.363	-0.090
Femur Neck	0.797 \pm 0.10	0.780 \pm 0.09	0.790 \pm 0.10	-2.133@	-0.878
Trochanter	0.720 \pm 0.11	0.713 \pm 0.12	0.688 \pm 0.07	-0.972	-4.44
Lumbar Sp	0.581 \pm 0.09	0.580 \pm 0.08	0.615 \pm 0.12	-0.172	5.85
Weights Plus					
Total Body	1.129 \pm 0.10	1.128 \pm 0.10	1.118 \pm 0.09	-0.089	-0.974
Femur Neck	0.862 \pm 0.13	0.858 \pm 0.15	0.859 \pm 0.13	-0.464	0.117
Trochanter	0.776 \pm 0.14	0.778 \pm 0.16	0.798 \pm 0.15	0.258	2.840
Lumbar Sp	0.658 \pm 0.15	0.632 \pm 0.12	0.680 \pm 0.12	-3.951	3.343
Weights Plus + Drug					
Total Body	1.05 \pm 0.05	1.036 \pm 0.05	1.043 \pm 0.05	-0.952#	-0.667@
Femur Neck	0.762 \pm 0.07	0.763 \pm 0.07	0.763 \pm 0.08	0.131	0.131
Trochanter	0.669 \pm 0.09	0.680 \pm 0.08	0.688 \pm 0.07	1.644	2.840
Lumbar Sp	0.564 \pm 0.10	0.563 \pm 0.14	0.544 \pm 0.09	0.177	-3.546

Values are expressed as mean \pm standard deviation of hydroxyapatite g/cm² and % change. @p<0.05, #p<0.01

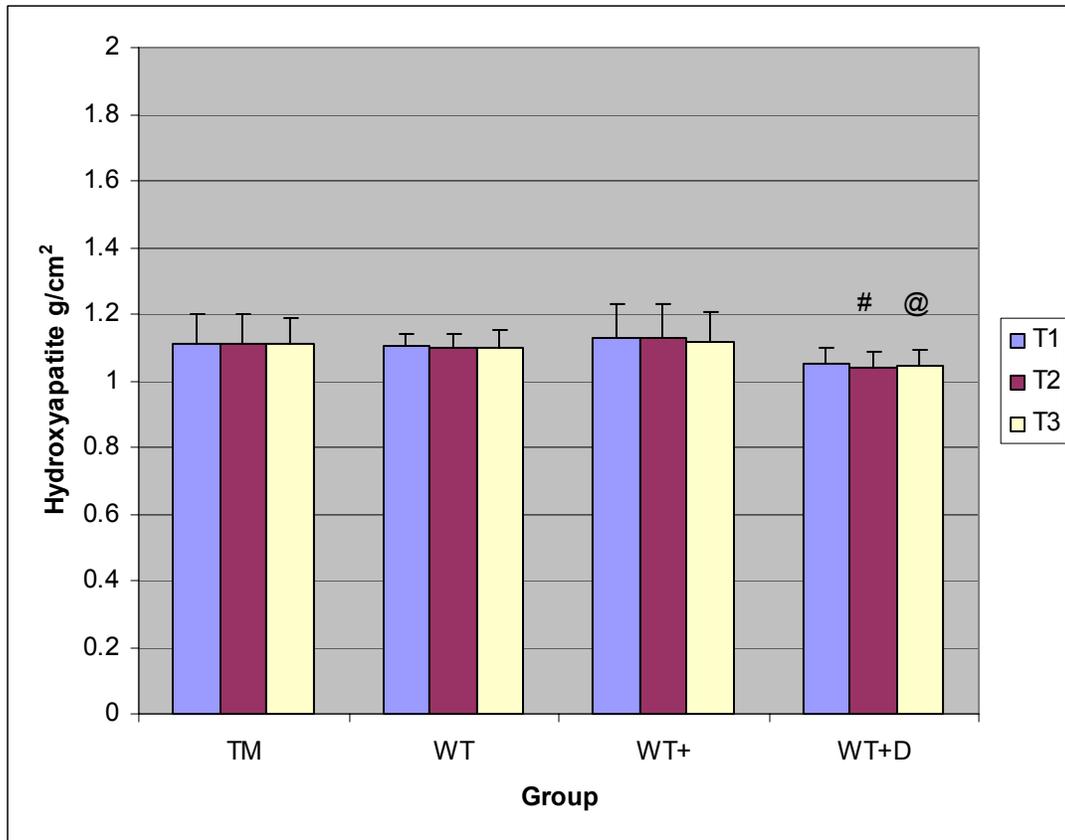


Figure 4-1. Absolute Total Body Bone Mineral Density. Values are means + SD Hydroxyapatite g/cm². TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ T3 BMD <T1, p<0.05, # T2 BMD <T1, p<0.01.

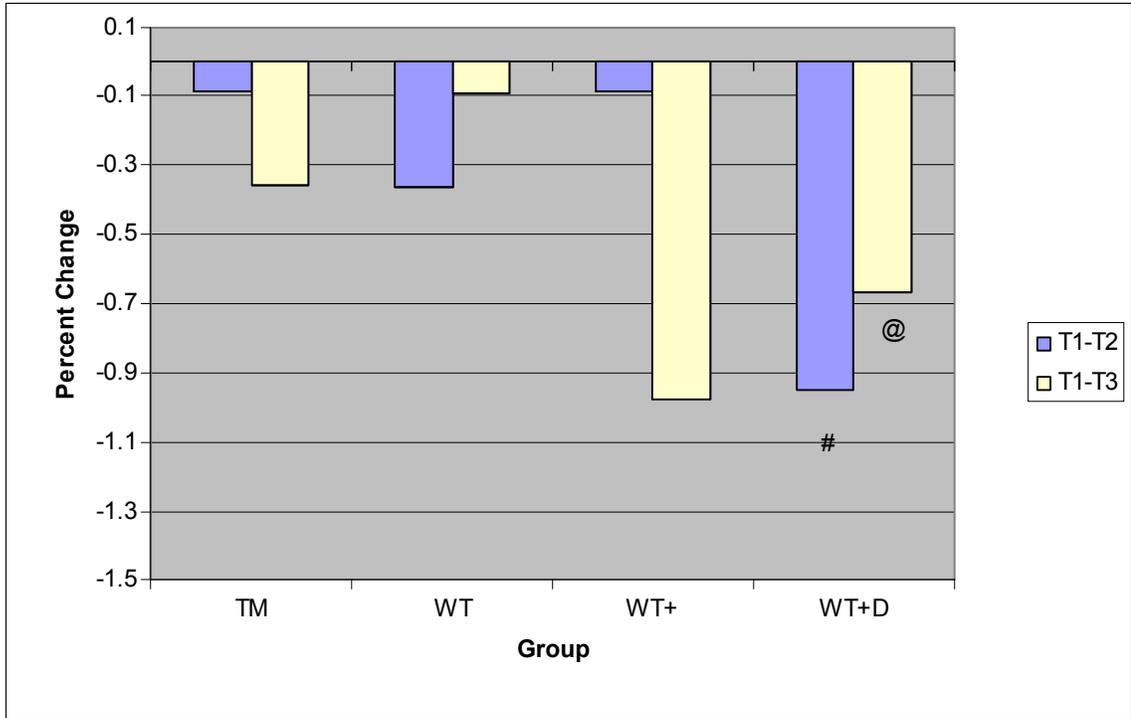


Figure 4-2. Relative Change in Total Body Bone Mineral Density. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ T3 BMD <T1, $p<0.05$, # T2 BMD <T1, $p<0.01$.

Femur Neck Bone Mineral Density

The means and standard deviations for femur neck bone mineral density are contained in Table 4-2. Absolute changes in BMD are shown in Figure 4-3. Relative changes in femur neck BMD are shown in Figure 4-4. The only significant change in BMD at the Femur neck occurred in the Weights group. This group had a significant loss of bone at T2 (-2.133% ($p<0.05$) when compared to T1 values

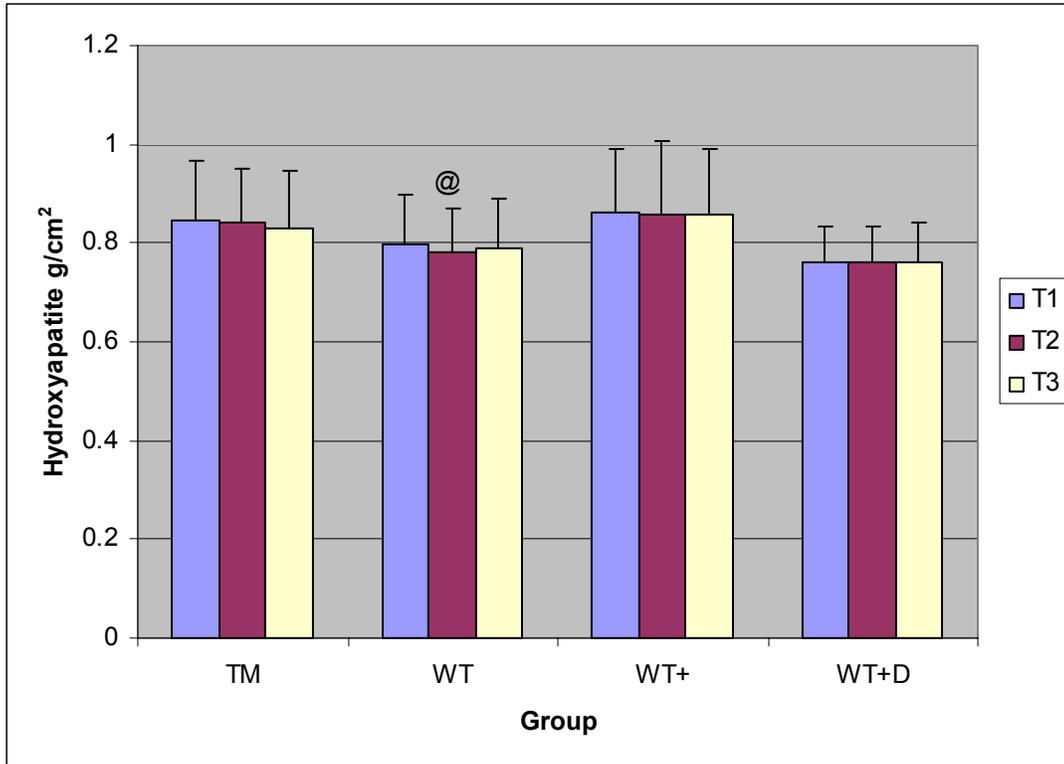


Figure 4-3. Absolute Femur Neck Bone Mineral Density. Values are mean + standard deviation Hydroxyapatite g/cm². WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ T2 BMD < T1, p < 0.05

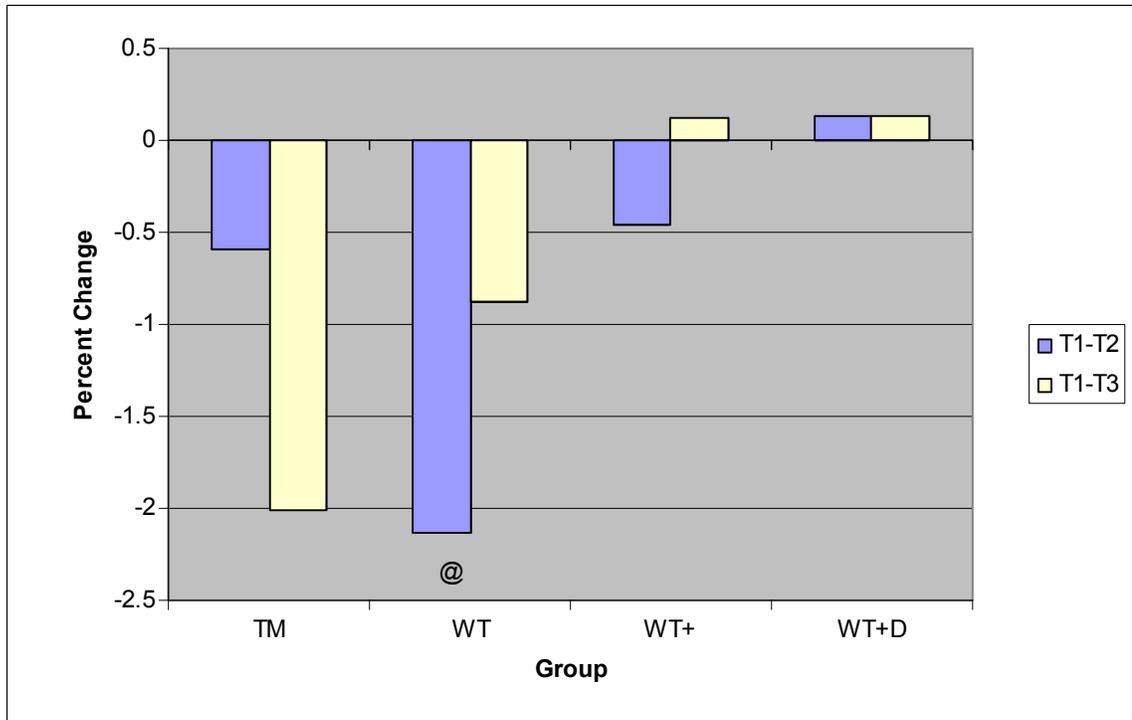


Figure 4-4 Relative Change in Femur Neck BMD. Values are % change.
 TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group,
 WT+D=Weights Plus and Drug Group. @ T2 BMD <T1, $p < 0.05$

Trochanter Bone Mineral Density

The means and standard deviations for trochanter bone mineral density are contained in Table 4-2. Absolute changes in trochanter BMD are shown in Figure 4-5. Relative changes in trochanter BMD are shown in Figure 4-6. There were no significant changes in either absolute or relative BMD of the trochanter for any group at either T2 or at T3. We do, however, detect a trend indicating that use of the Leg Press exercise by both the Weight Plus and the Weight Plus + Drug increased trochanter BMD.

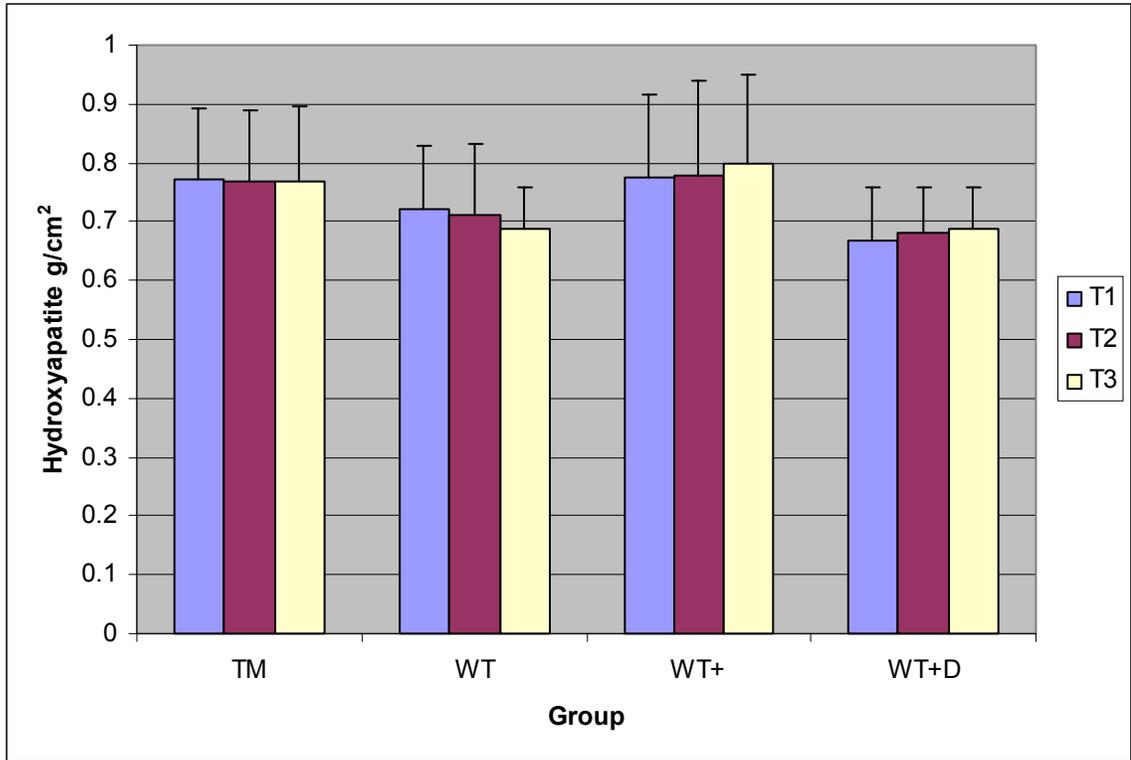


Figure 4-5. Absolute Trochanter Bone Mineral Density. Values are mean \pm standard deviation Hydroxyapatite g/cm². TM= Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

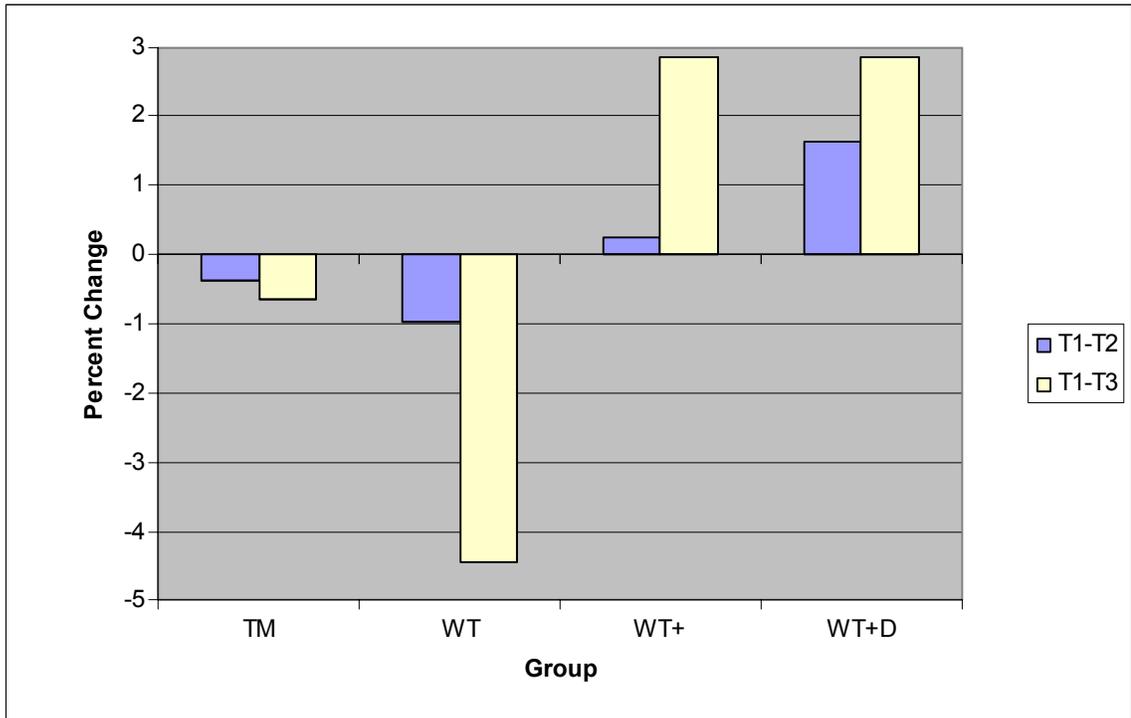


Figure 4-6. Relative Change in Trochanter Bone Mineral Density. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Lumbar Spine Bone Mineral Density

The means and standard deviations for lumbar spine bone mineral density are contained in Table 4-2. Absolute changes in Lumbar BMD are shown in Figure 4-7.

Relative changes in lumbar spine BMD are shown in Figure 4-8. There were no significant changes in BMD of the lumbar spine for any group between either T1-T2 or T1-T3.

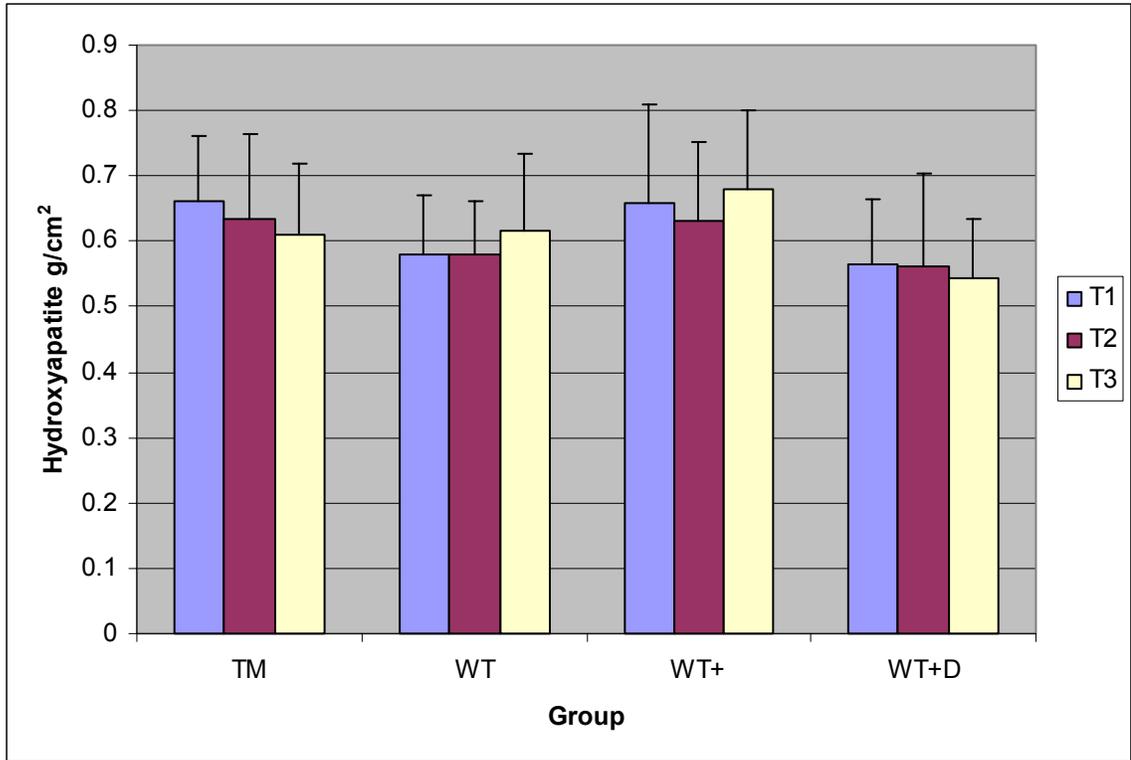


Figure 4-7. Absolute Lumbar Spine Bone Mineral Density. Values are mean \pm standard deviation Hydroxyapatite g/cm². TM= Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

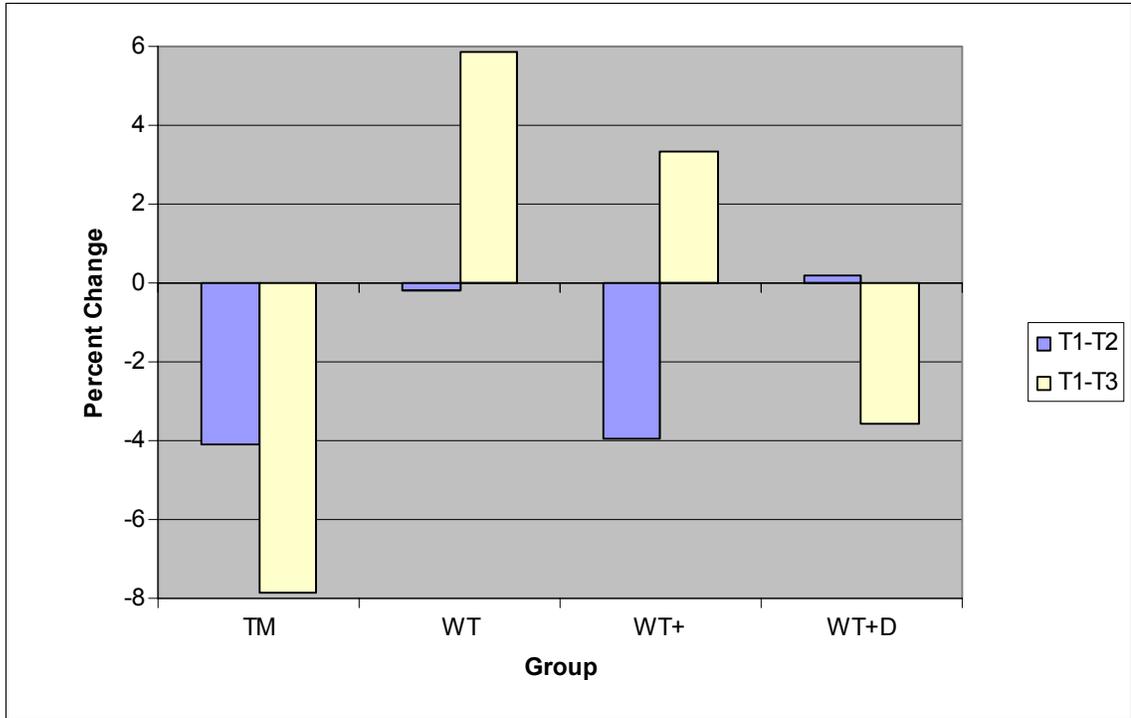


Figure 4-8. Relative Change in Lumbar Spine Bone Mineral Density. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Serum Markers of Bone Metabolism

At T1, T2, and T3, venous blood samples were drawn from 46 subjects. Blood samples were processed immediately and stored at -80 degrees centigrade. Samples for all biochemical markers were assayed in a single lot at the end of the study and assays were done in duplicate.

Table 4-3. Serum Markers of Bone Metabolism

Treadmill	T1	T2	T3	% Chg T1-T2	% Chg T2-T3	% Chg T1-T3
Osteocalcin	25.21 \pm 3.6	25.79 \pm 3.2	24.57 \pm 3.5	2.30	-4.73	-2.52
Pyridinoline	1.90 \pm 0.67	2.038 \pm 0.73	2.27 \pm 0.59	7.26	11.38@	19.47@
Weights						
Osteocalcin	23.31 \pm 3.9	23.39 \pm 2.8	23.50 \pm 4.0	0.34	0.47	0.82
Pyridinoline	1.64 \pm 0.40	1.60 \pm 0.27	1.85 \pm 0.44	-2.4	15.63@	12.8
Weights Plus						
Osteocalcin	26.04 \pm 5.19	26.20 \pm 4.35	25.80 \pm 4.5	-0.61	-1.53	-0.92
Pyridinoline	1.97 \pm 0.33	2.10 \pm 0.41	1.84 \pm 0.45	6.60	-12.40	-6.60
Weights Plus + Drug						
Osteocalcin	20.55 \pm 3.9	21.40 \pm 3.2	18.67 \pm 7.1	4.14	-12.76	-9.1
Pyridinoline	1.58 \pm 0.50	1.51 \pm 0.56	1.69 \pm 0.58	-4.4	11.92@	7.0

Values are expressed as the mean \pm standard deviation. Values for Osteocalcin are expressed as ng/ml. Values for pyridinoline are expressed as nmol/L @ p<0.05,

Osteocalcin

The means and standard deviations for all serum markers of bone metabolism are contained in Table 4-3. At T1 there were significant differences only between the

Weight Plus and Weight Plus + Drug groups (26.04 to 20.55, p<0.05). There were no significant differences between any of the groups at T1-T2, T2-T3, or at T1-T3.

Absolute changes in osteocalcin are shown in Figure 4-9. Relative changes in osteocalcin

are shown in Figure 4-10. At T2 there were significant differences in osteocalcin

between the Weight Plus + Drug and Treadmill (26.20 to 25.79, p<0.05) group and

between the Weights Plus and the Weights Plus + Drug groups (26.20 to 21.40, p<0.01).

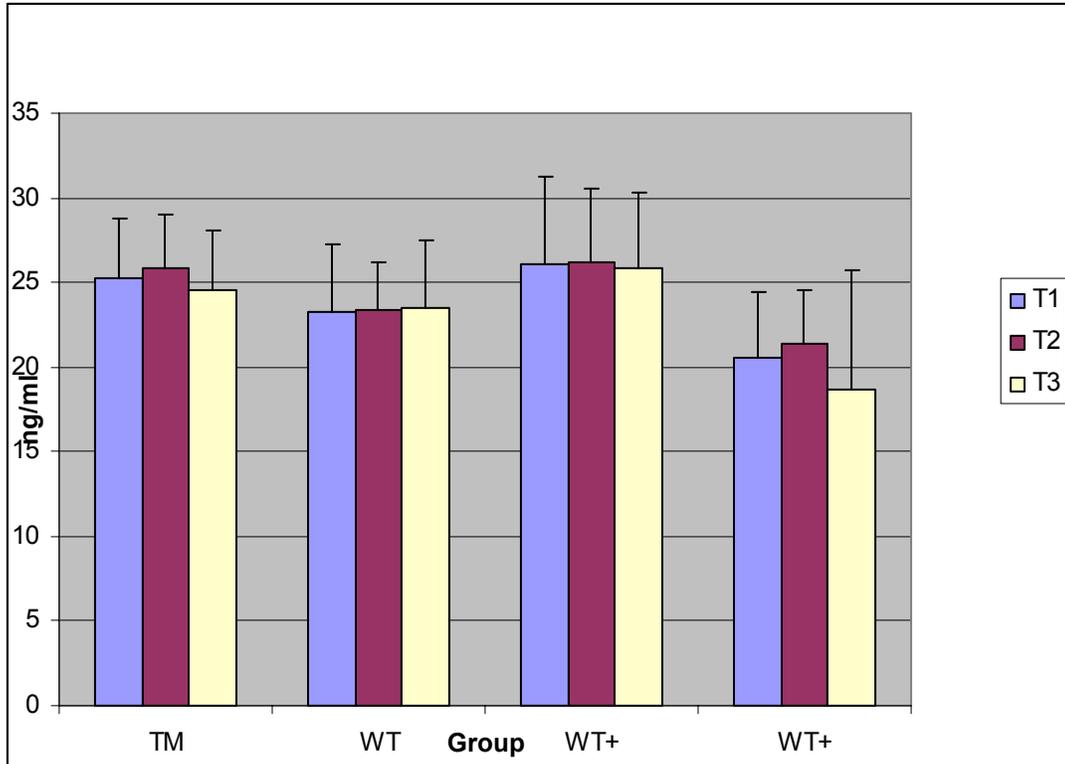


Figure 4-9. Absolute Serum Osteocalcin Levels (ng/ml). Values are mean \pm standard deviation. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

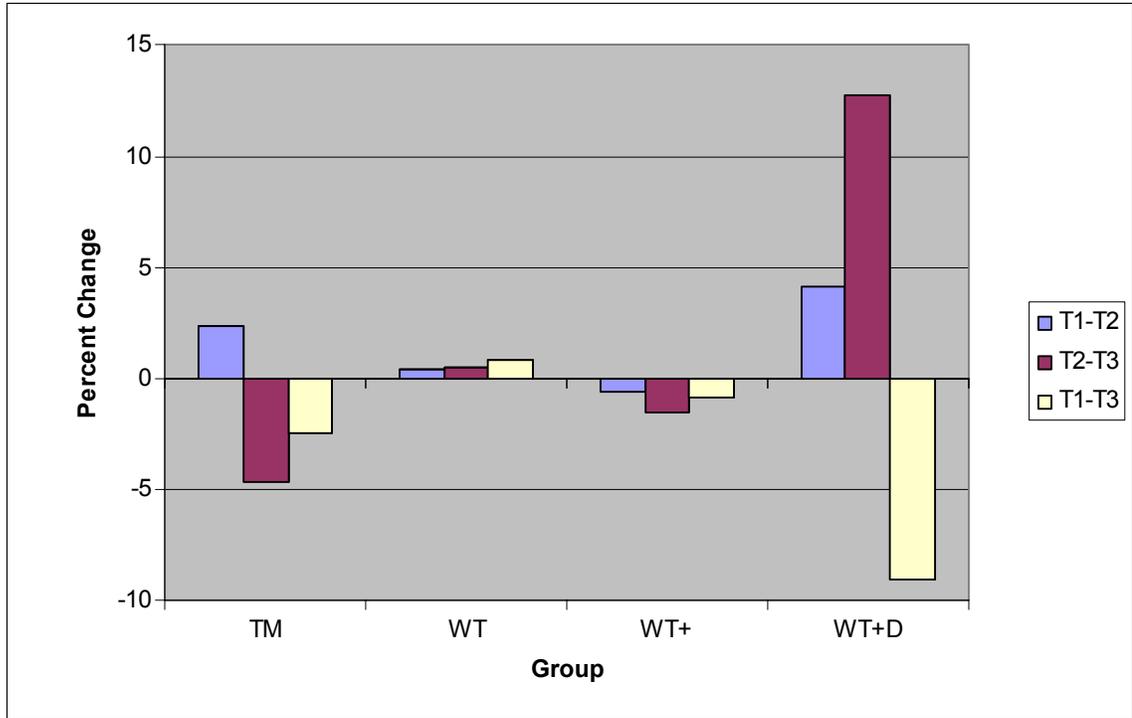


Figure 4-10. Relative Change in Serum Osteocalcin Levels. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Pyridinoline Crosslinks (PYD)

Pyridinoline is a marker of bone resorption. Normal PYD levels are 1.5-2.0 nmol/L. Pyridinoline levels were measured in the serum of 46 subjects using a Metra Serum PYD EIA kit (Quidel Corporation, San Diego, CA). When bone is degraded PYD is released into circulation. The means and standard deviations for serum pyridinoline are contained in Table 4-3. Absolute changes in PYD are shown in Figure 4-11. Relative changes in PYD are shown in Figure 4-12. There were no significant changes in pyridinoline levels for any group from T1-T2. There were significant differences at T2-T3 in the Treadmill group (11.38%, $p<0.05$), and the Weight Plus + Drug group (11.92%, $p<0.05$) The only significant change from T1-T3 was found in the Treadmill group which experienced a 19.47% increase in serum PYD ($p<0.05$).

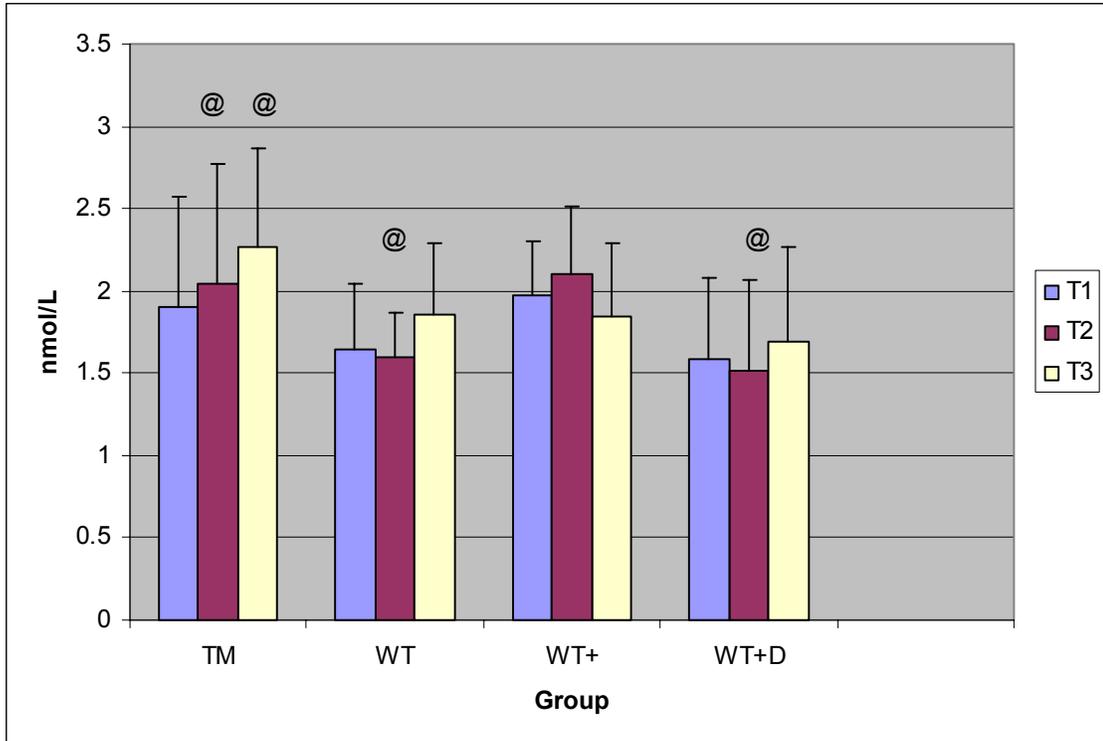


Figure 4-11. Absolute Serum Pyridinoline Levels (nmol/L). Values are mean \pm standard deviation. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ T3>T1, $p<0.05$.

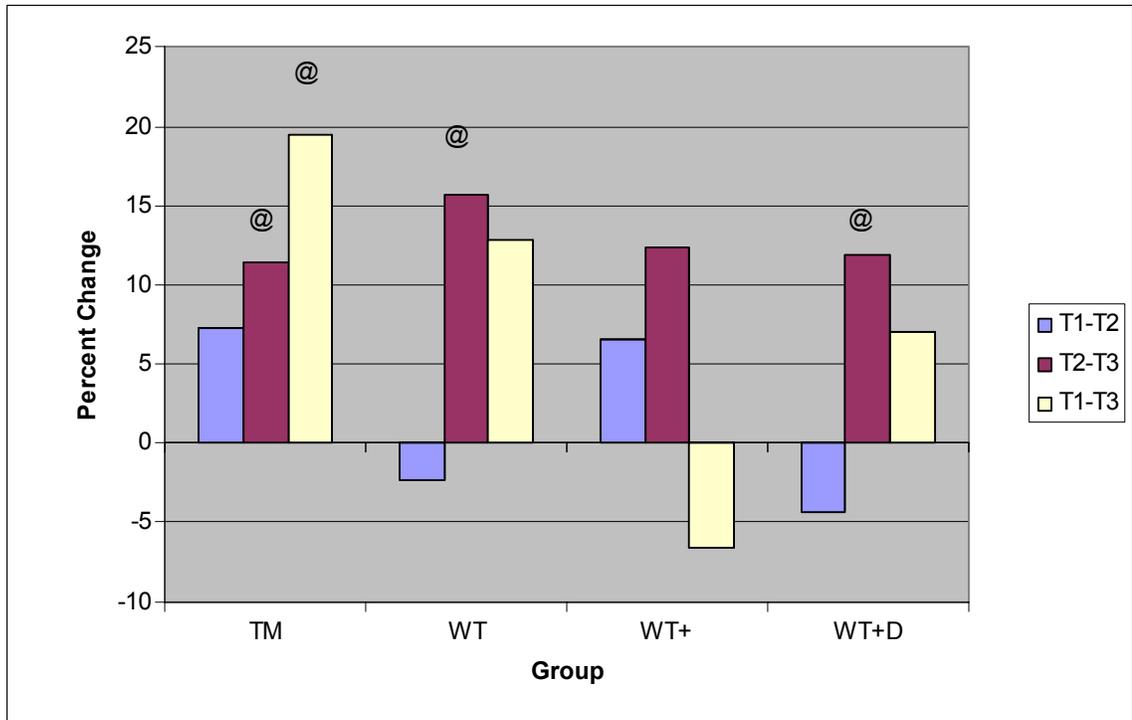


Figure 4-12. Relative Changes in Serum Pyridinoline Levels. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ T3>T1, p<0.05..

Body Composition

At T1, T2, and T3 measurements of body composition were performed using DXA on the 46 women participating in the study. There were no statistically significant differences between the groups at the start of the study. Body composition variables measured included:

- Body Mass (kg)
- Fat (kg)
- Fat Percent
- Lean Mass (kg)
- Bone Mineral Content (BMC) (kg)

Body Mass

The means and standard deviations for body mass are contained in Table 4-4.

Absolute changes in body mass are shown in Figure 4-13. Relative changes in body mass

are shown in Figure 4-14. There were no significant changes in body mass among groups during the course of the study.

Table 4-4. Body Composition

	T1	T2	T3	% Chg T1-T2	% Chg T1-T3
Treadmill					
Wt (kg)	70.090±14.745	68.597±15.234	69.748±15.045	-2.13	-0.488
Fat (%)	42.238±8.968	41.885±8.05	42.008±7.63	-0.835	-0.545
Fat (kg)	29.178±10.847	27.973±11.679	28.001±11.30	-4.130	-4.034
Lean Mass (kg)	37.911±5.561	36.737±8.161	36.973±8.38	-3.096	-2.474
BMC (kg)	2.281±0.389	2.163±0.501	2.195±0.577	-5.173	-3.777
Weights					
Wt (kg)	64.644±7.342	64.469±6.788	64.060±6.714	-0.271	-0.903
Fat (%)	42.608±5.113	41.617±4.276	41.125±4.91	-2.326	-3.480
Fat (kg)	27.631±6.684	25.945±4.407	25.607±4.833	-6.102	-7.325
Lean Mass (kg)	35.739±3.959	36.031±3.904	36.336±3.975	0.817	1.670
BMC (kg)	2.140±0.136	2.155±0.202	2.156±0.236	0.701	0.748
Weights Plus					
Wt (kg)	71.015±14.200	70.848±14.552	71.315±16.65	-0.235	0.422
Fat (%)	39.733±8.052	39.167±6.721	39.383±7.77	-1.425	-0.881
Fat (kg)	27.867±11.339	27.492±10.698	26.432±14.51	-1.346	-5.149
Lean Mass (kg)	40.124±5.070	40.566±5.484	40.699±5.388	1.102	1.433
BMC (kg)	2.389±.404	2.368±.403	2.339±0.35	0.879	-2.093
Weights Plus + Drug					
Wt (kg)	63.70±5.589	64.109±5.207	64.009±5.125	0.642	0.485
Fat (%)	38.650±6.230	39.170±5.459	38.85±7.7330	1.345	0.517
Fat (kg)	23.697±5.860	24.565±4.983	24.291±5.319	3.663	2.507
Lean Mass (kg)	36.898±2.426	37.218±2.789	37.603±2.785	0.867	1.812
BMC (kg)	1.980±.162	1.959±.132	1.978±0.159	-1.061	-0.101

Values are expressed as mean ± standard deviation

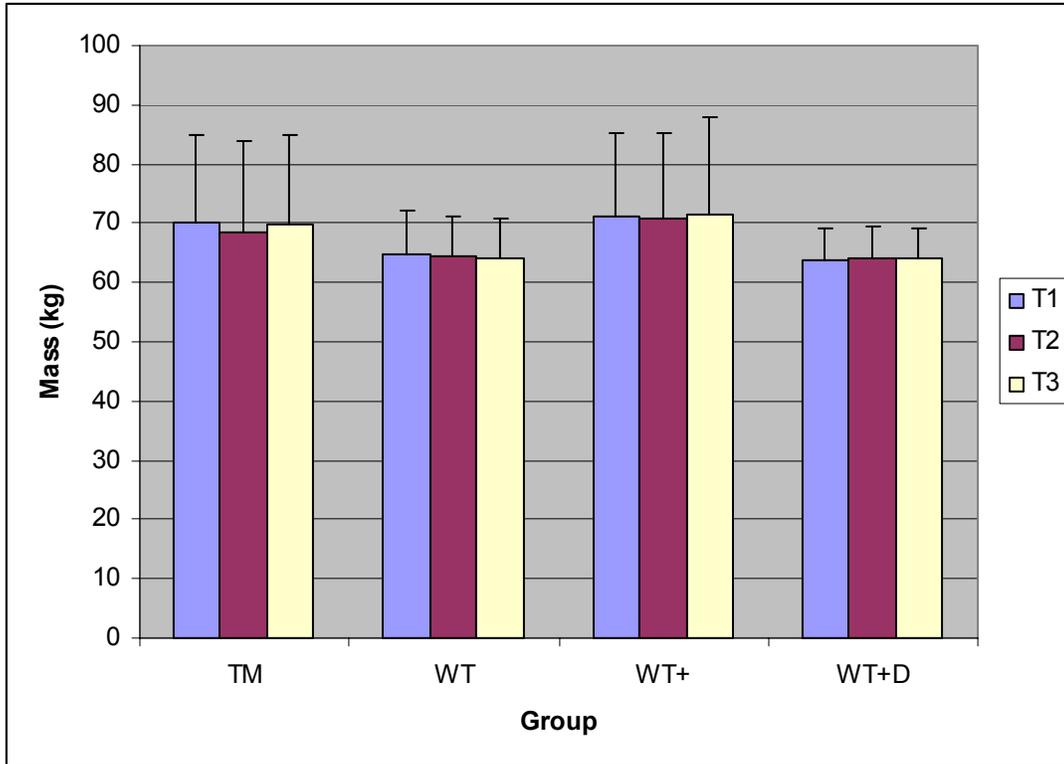


Figure 4-13. Absolute Body Mass (kg). Values are mean + standard deviation.
TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group,
WT+D=Weights Plus and Drug Group.

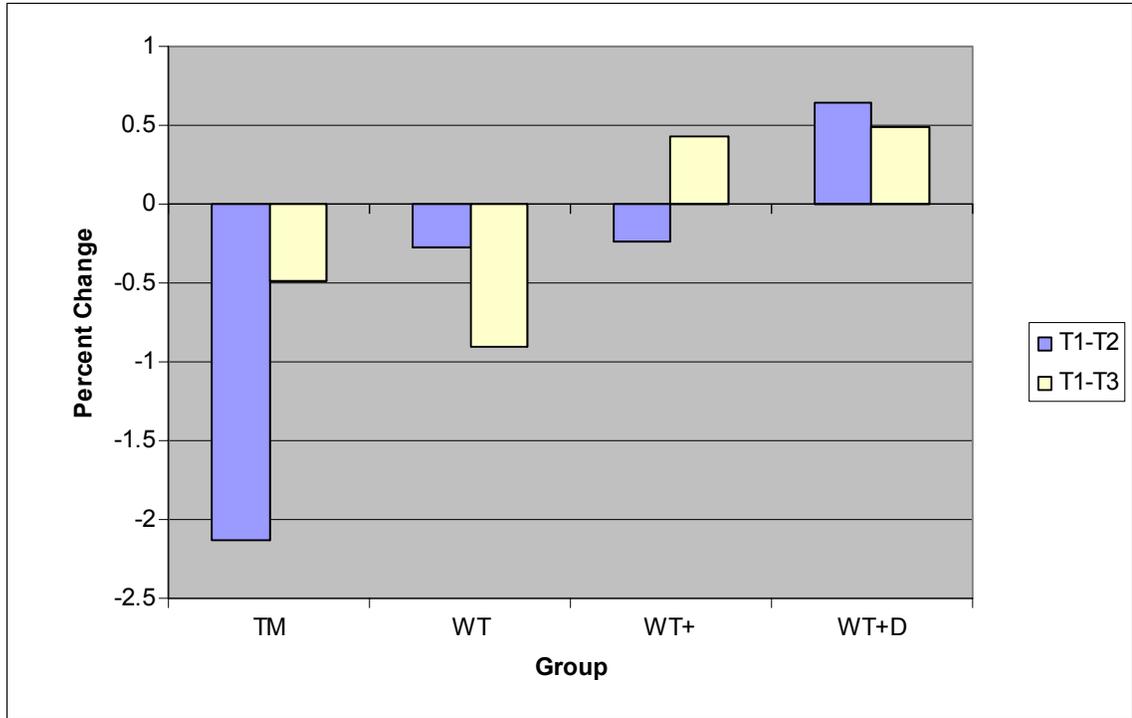


Figure 4-14. Relative Changes in Body Mass. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Fat Mass and Fat Percentage

The means and standard deviations for fat mass and relative body fat are contained in Table 4-4. Absolute changes in fat mass are shown in Figure 4-15. Relative changes in fat mass are shown in Figure 4-16. There were no significant changes in fat mass or relative fat percent for any of the groups during the study.

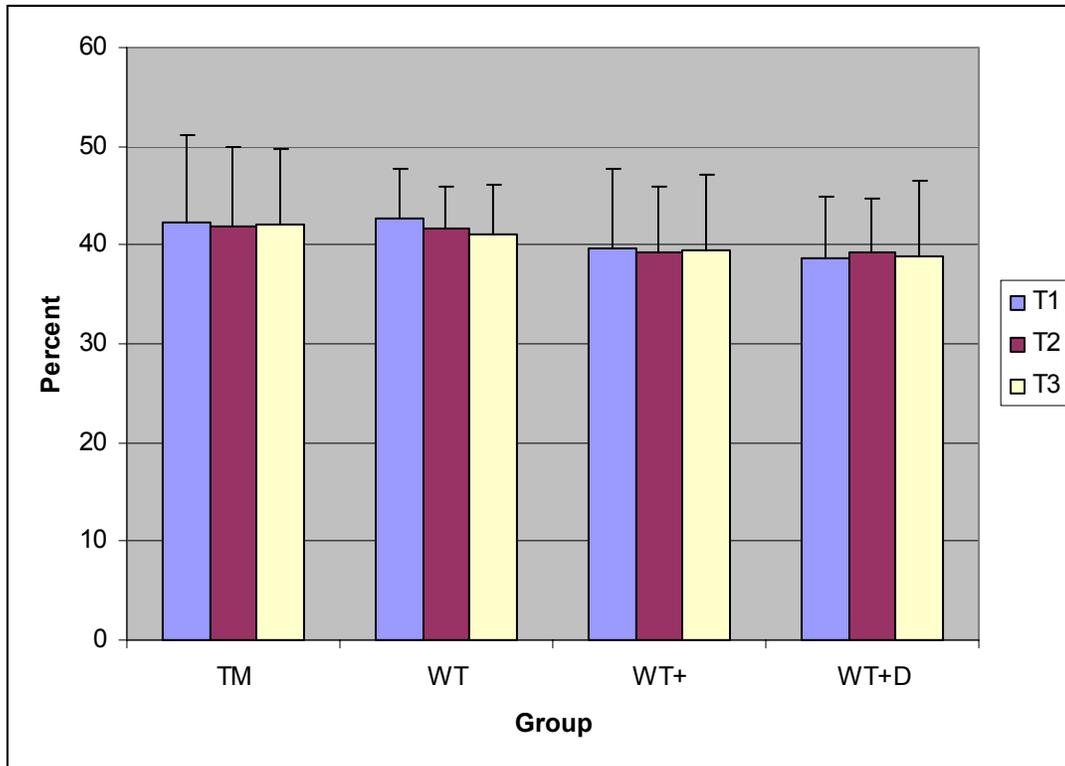


Figure 4-15. Changes in Percent Body Fat. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

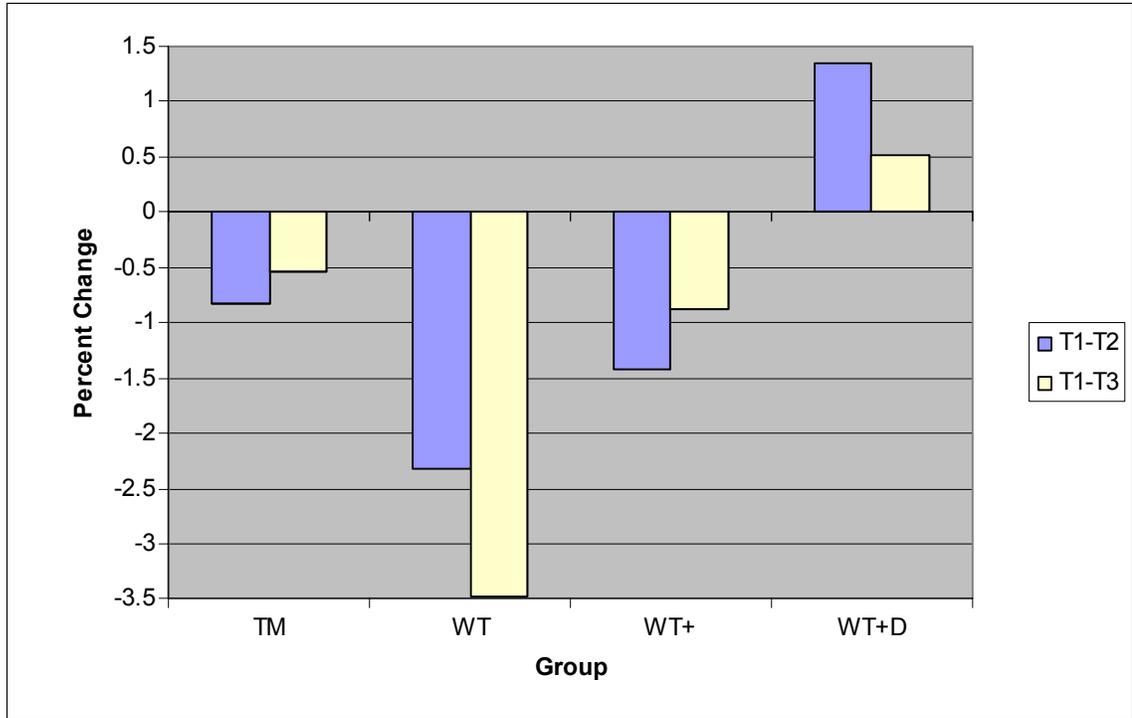


Figure 4-16. Relative Changes in Fat Percentage. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Lean Mass

The means and standard deviations for Lean Tissue Mass are contained in Table 4-4. Absolute changes in Lean Tissue Mass are shown in Figure 4-17. Relative changes in Lean Tissue mass are shown in Figure 4-18. There were no significant changes Lean Tissue for any of the groups during the study.

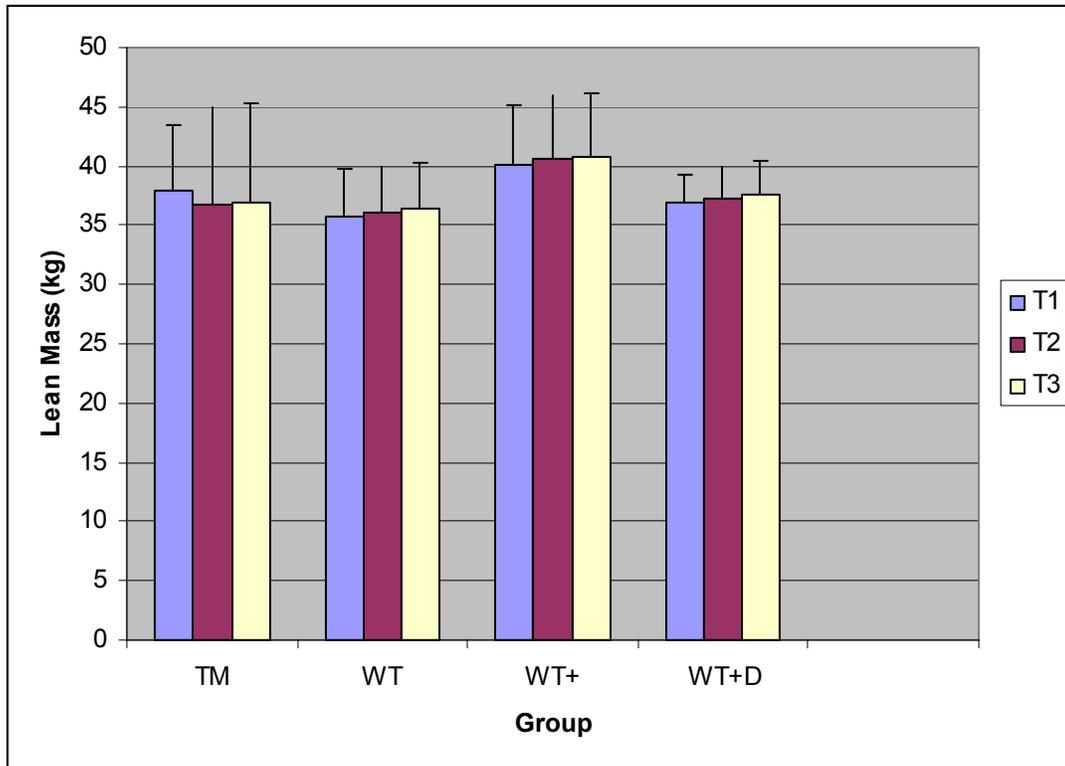


Figure 4-17. Absolute Lean Tissue (kg). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

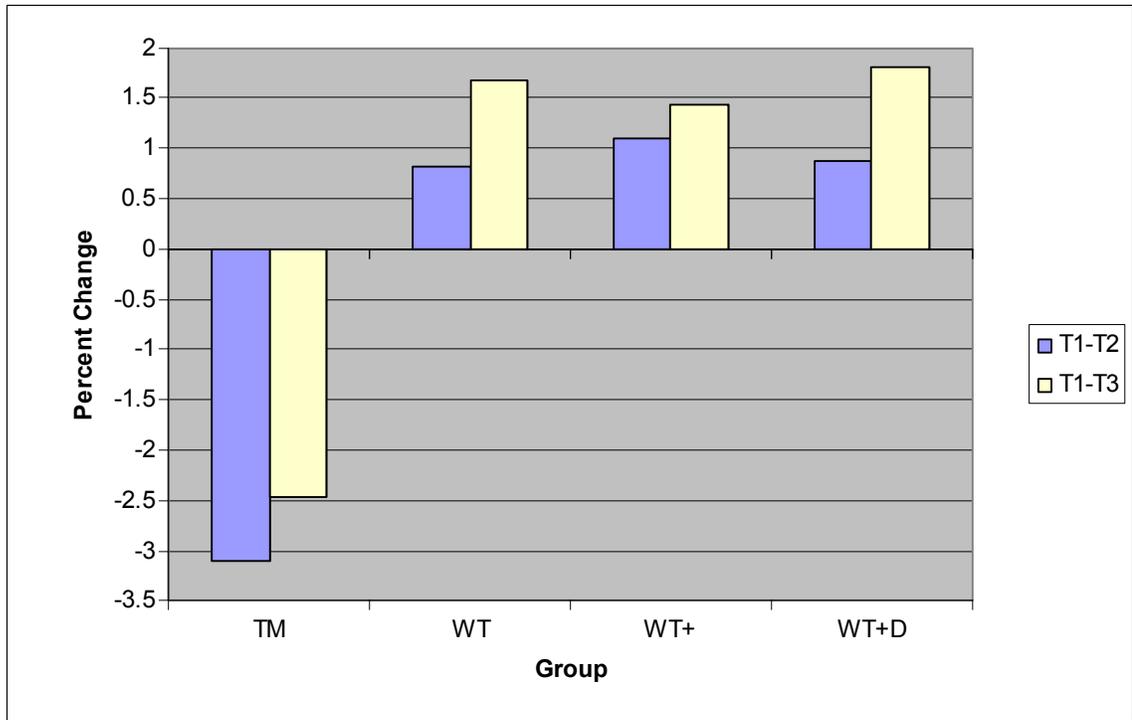


Figure 4-18. Relative Changes in Lean Tissue Mass. Values are % change.
 TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group,
 WT+D=Weights Plus and Drug Group.

Bone Mineral Content

Bone mineral content (BMC) represents the amount of hydroxyapatite present in bone tissue without regard for the volume of bone present. The means and standard deviations for Bone Mineral Content (BMC) are contained in Table 4-4. Absolute changes in BMC are shown in Figure 4-19. Relative changes in BMC are shown in Figure 4-20. There were no significant changes BMC for any of the groups during the study, although there appears to be a trend suggesting that the resistance training subjects gained more in Lean Tissue Mass than did the Treadmill group.

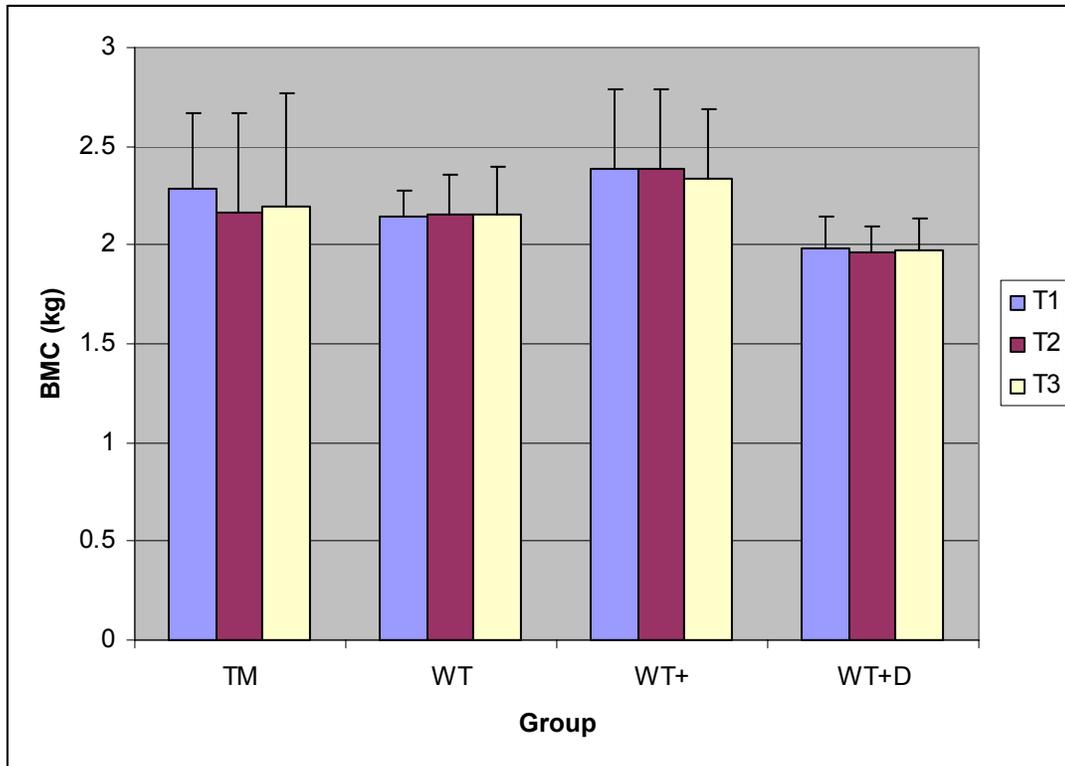


Figure 4-19. Absolute Total Bone Mineral Content (kg). Values are mean \pm standard deviation (kg). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.

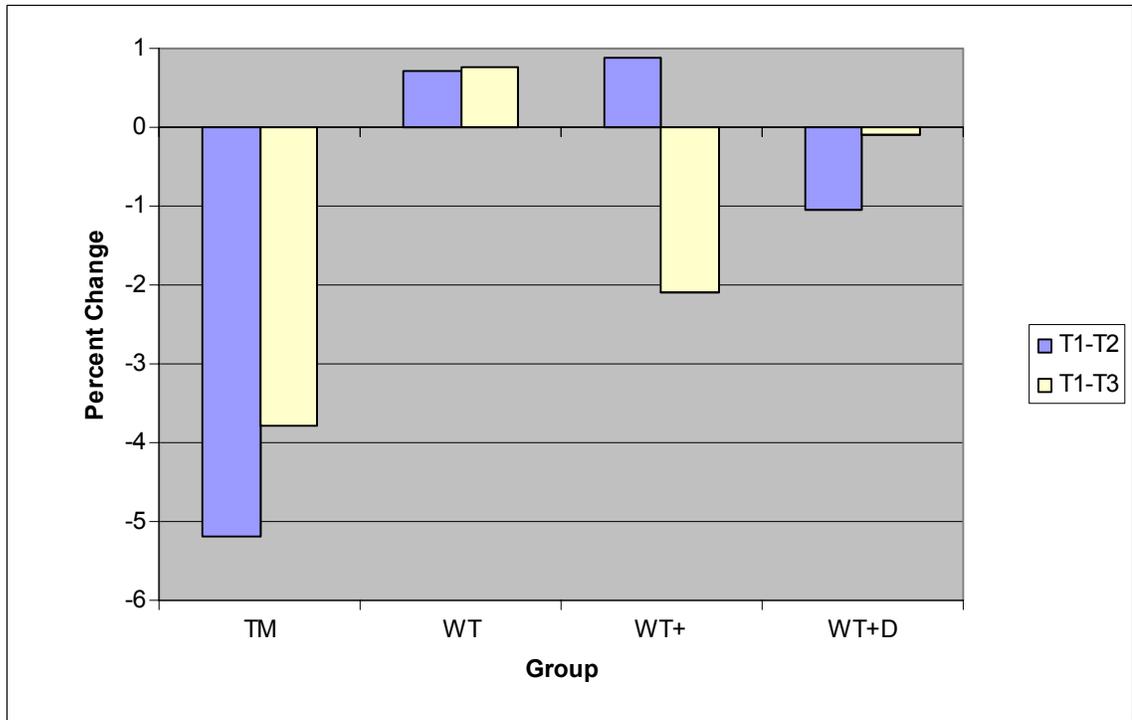


Figure 4-20. Relative changes in Total Bone Mineral Content. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group

Strength Testing

Strength testing using an isometric lumbar extension machine (MedX) and one—repetition maximum (1-RM) testing was conducted on all study participants at baseline. There was no statistically significant difference between the individuals assigned to the four study groups at the beginning of the study. Strength testing was repeated after 4.5 and 9 months of exercise intervention.

MedX Lumbar Extensor Strength Testing

The MedX Lumbar Extensor machine permits a multi-angle isometric test of lumbar extensor strength. Each angle represents degrees of lumbar flexion, up to a maximum of 60°. The means and standard deviations for lumbar extensor muscle strength are contained in Table 4-5.

In the Treadmill group, the only significant change in lumbar extensor strength occurred between T1 and T2 where this group registered a 7.47% ($p < 0.05$) increase in strength at 60° angle of lumbar flexion. The Weights group had significant strength increases five of the six angles measured, but not at 24° of flexion at T1-T2. Significant increases at T2 included: 0° (64.48%, $p < 0.05$), 12° (14.23%, $p < 0.05$), 36° (9.25%, $p < 0.05$), 48° (6.13%, $p < 0.05$), and 60° (4.83, $p < 0.05$). At T3, the Weights group had significant changes compared to baseline at all angles: 0 degrees (74.92%, $p < 0.01$), 12 degrees (25.18, $p < 0.05$), 24 degrees 27.97%, $p < 0.01$) 36 degrees (21.46%, $p < 0.01$), 48 degrees (12.63%, $p < 0.01$), 60 degrees (10.91%, $p < 0.01$)

The Weights Plus group's training protocol specifically targeted the lumbar extensor muscles and this group had significant strength gains at every angle of the isometric lumbar extension strength test. Between T1 and T2, significant strength gains occurred at 0° (64.66%, $p < 0.01$), 12° (45.82%, $p < 0.05$), 24° (39.12%, $p < 0.05$), 36° (37.69%, $p < 0.05$), 48° (32.44%, $p < 0.05$), and 60° (16.23%, $p < 0.05$). At T3, the Weights Plus group showed even greater strength gains over baseline. These changes include increases over baseline at 0° (86.73%, $p < 0.01$), 12° (62.0%, $p < 0.05$), 24° (56.64%, $p < 0.05$), 36° (49.33%, $p < 0.01$), 48° (46.66%, $p < 0.01$) and 60° (30.86%, $p < 0.01$).

The Weights Plus + Drug group showed significant gains between T1 and T2 at 0° (90.13%, $p < 0.01$), 12° (68.18%, $p < 0.001$), 24° (48.38%, $p < 0.01$) and 36° (28.13%, $p < 0.05$). Strength gains continued throughout the study and at T3 significant increases for this group were seen at all six measurement positions: 0° (108.20, $p < 0.01$), 12° (75.82%, $p < 0.001$), 24° (49.58%, $p < 0.001$), 36° (31.72%, $p < 0.05$), 48° (26.58%, $p < 0.05$) and 60° (25.96%, $p < 0.01$)

Table 4-5. MedX Lumbar Extension 6-Angle Isometric Test. Values are foot-pounds expressed as mean \pm standard deviation. @p<0.05 #p<0.01 \$p<0.001.

	T1	T2	T3	% Change T1-T2	% Change T1-T3
Treadmill					
0 Degrees	56.33 \pm 44.42	61.08 \pm 48.89	67.58 \pm 56.86	8.43	19.97
12 Degrees	84.33 \pm 54.55	87.00 \pm 56.23	94.25 \pm 51.91	3.17	11.76
24 Degrees	101.75 \pm 63.17	105.17 \pm 67.15	109.58 \pm 59.87	3.36	7.70
36 Degrees	110.33 \pm 51.14	125.50 \pm 60.67	119.75 \pm 47.37	13.75	8.54
48 Degrees	132.48 \pm 41.94	143.83 \pm 54.97	145.5 \pm 56.79	8.57	9.83
60 Degrees	140.50 \pm 41.10	151.00 \pm 47.87	152.67 \pm 55.95	7.47@	8.66
Weights					
0 Degrees	35.92 \pm 27.16	59.08 \pm 34.77	62.83 \pm 33.55	64.48@	74.92#
12 Degrees	68.50 \pm 34.60	78.25 \pm 31.67	85.75 \pm 35.67	14.23@	25.18@
24 Degrees	81.33 \pm 36.22	89.33 \pm 30.62	104.08 \pm 32.97	9.84	27.97#
36 Degrees	92.83 \pm 34.56	101.42 \pm 34.88	112.75 \pm 33.70	9.25@	21.46#
48 Degrees	111.50 \pm 22.39	118.33 \pm 34.27	125.58 \pm 30.44	6.13@	12.63#
60 Degrees	120.75 \pm 25.70	126.58 \pm 41.02	133.92 \pm 30.46	4.83@	10.91#
Weights Plus					
0 Degrees	70.25 \pm 66.33	115.67 \pm 33.75	131.18 \pm 40.54	64.66#	86.73#
12 Degrees	102.75 \pm 77.51	149.83 \pm 43.56	166.45 \pm 52.73	45.82@	62.00@
24 Degrees	117.58 \pm 76.34	163.58 \pm 69.25	184.18 \pm 60.65	39.12@	56.64@
36 Degrees	123.83 \pm 73.61	170.5 \pm 60.29	184.91 \pm 65.82	37.69@	49.33#
48 Degrees	137.67 \pm 72.70	182.33 \pm 51.11	201.91 \pm 60.23	32.44@	46.6#
60 Degrees	158.67 \pm 64.38	184.42 \pm 50.18	207.64 \pm 71.45	16.23@	30.86#
Weights Plus + Drug					
0 Degrees	52.30 \pm 39.09	99.44 \pm 31.48	108.89 \pm 37.61	90.13#	108.20#
12 Degrees	77.10 \pm 41.39	129.67 \pm 20.88	135.56 \pm 36.39	68.18\$	75.82\$
24 Degrees	93.30 \pm 38.11	138.44 \pm 19.67	139.56 \pm 32.19	48.38#	49.58\$
36 Degrees	108.40 \pm 32.20	138.89 \pm 18.09	142.78 \pm 33.07	28.13@	31.72@
48 Degrees	122.10 \pm 34.72	142.00 \pm 21.02	154.56 \pm 34.54	16.30	26.58@
60 Degrees	131.7 \pm 33.76	152.22 \pm 28.00	165.89 \pm 32.70	15.58	25.96#

Values are expressed as mean \pm standard deviation. Values are expressed in foot-pounds, @p<0.05 #p<0.01 \$p<0.001

MedX Lumbar Extension Strength Index

The strength index is a proprietary relative measure of lumbar extensor strength generated by the MedX lumbar extension machine software program. It was obtained for all groups and the results are contained in Table 4-6. Absolute changes in Strength Index

appear in Figure 4-21. Relative changes in Strength Index are in Figure 4-22. Only the Weights Plus and Weights Plus + Drug groups routinely trained on the lumbar extension machine, and these were the only groups to show significant change at T2 (+38.98%, $p < 0.01$ and +29.56%, $p < 0.01$ respectively). At T3, the Weights (+20.86, $p < 0.05$) Weights Plus (+51.14%, $p < 0.01$) and the Weights Plus + Drug (+33.85%, $p < 0.01$) groups showed significant improvement in Strength Index compared to T1 values.

Table 4-6. MedX Strength Index (SI) by Group.

	T1	T2	T3	% Change T1-T2	% Change T1-T3
Treadmill	7557+3328	7944+3647	8180+3694	5.1	8.24
Weights	6351+1720	7169+1584	7676+1958	12.88	20.86@
Weights Plus	8163+3779	11345+2887	12338+3218	38.98#	51.14#
Weights Plus + Drug	7432+2515	9629+1671	9948+2577	29.56#	33.85#

Values are expressed as mean \pm standard deviation. @p<0.05 #p<0.01

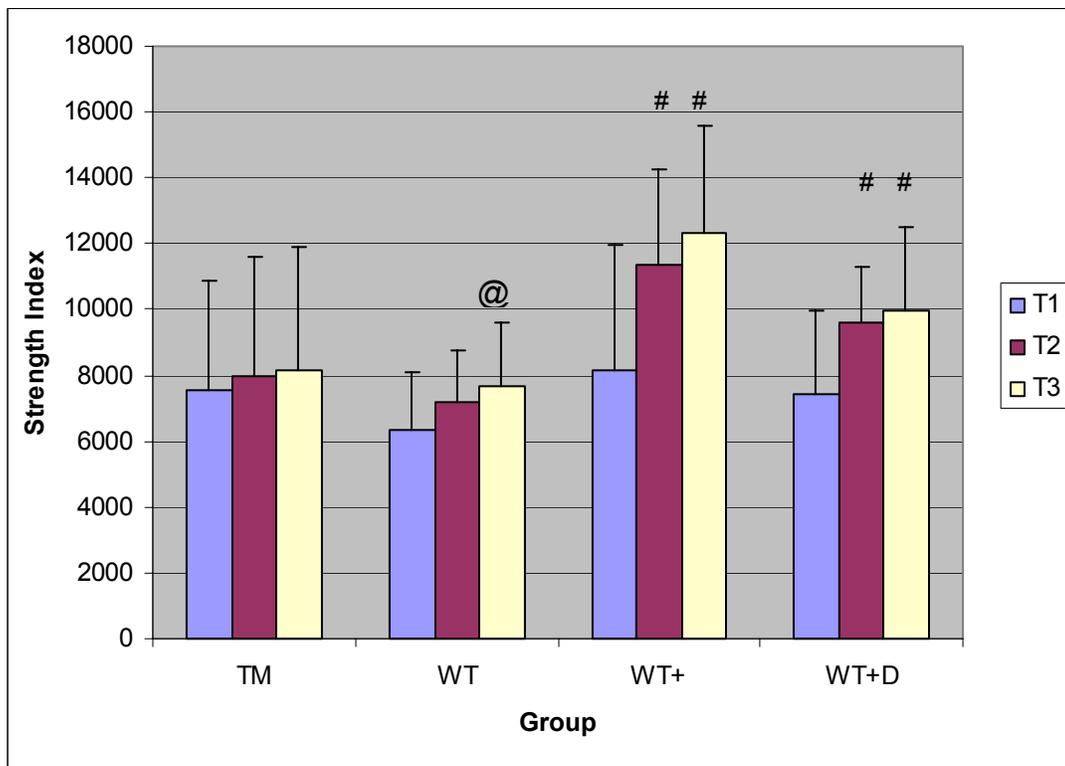


Figure 4-21. Absolute Changes in Lumbar Extensor Strength Index(SI). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @= p<0.05; # =p<0.01

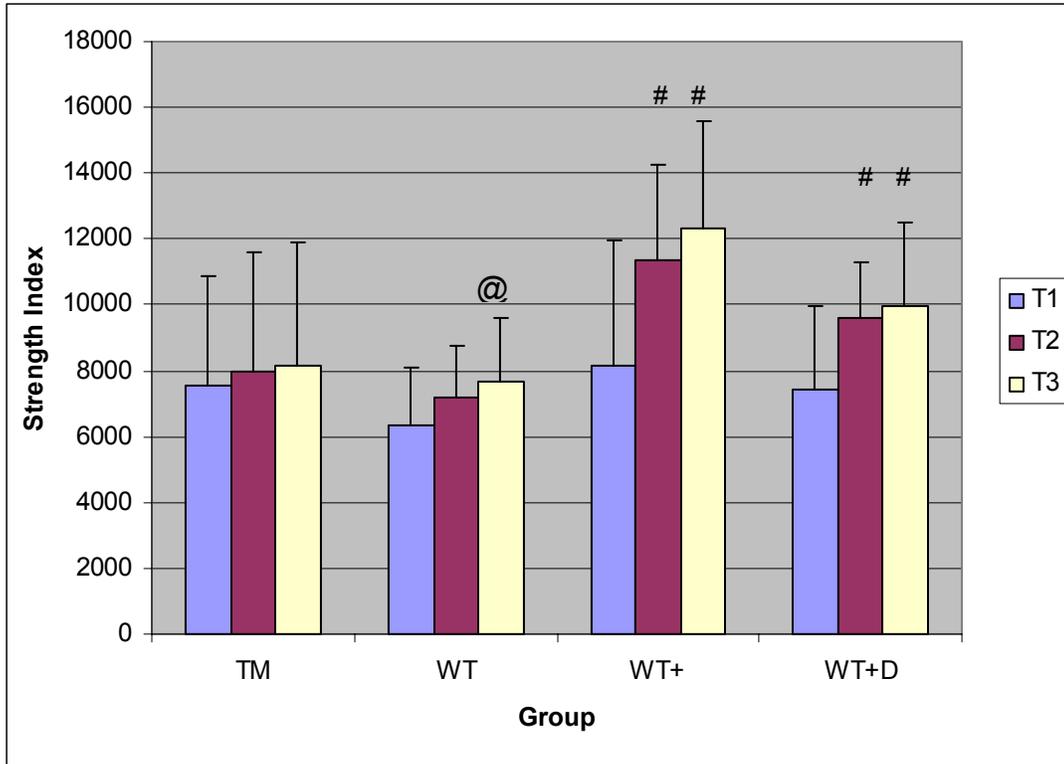


Figure 4-22. Relative Changes in Lumbar Strength Index. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @= $p < 0.05$; # = $p < 0.01$

1-Repetition Maximum Strength Testing

One Repetition-Maximum (1-RM) testing was performed on all 46 participants of the study. The results are contained in Table 4-7.

Table 4-7. 1RM Testing. Values are pounds expressed as mean \pm standard deviation.
 @p<0.05 #p<0.01 \$p<0.001. CP=Chest press TA Torso Arm OP =Overhead
 Press SR=Seated Row LE=Leg Extension LP=Leg Press

	T1	T2	T3	% Change T1-T2	% Change T1-T3
Treadmill					
Upper Body					
CP	111.38 \pm 25	115.38 \pm 30	117.38 \pm 31	3.53	5.39
TA	183.38 \pm 35.09	191.38 \pm 37.69	191.08 \pm 40.44	4.36@	4.20
OP	119.38 \pm 40.11	113.69 \pm 22.83	115.23 \pm 23.85	-4.77	-3.47
SR	166.15 \pm 30.75	167.54 \pm 37.15	180.46 \pm 29.90	0.84	8.61@
Lower Body					
LE	151.38 \pm 39.3	152.92 \pm 36.0	154.92 \pm 37.7	0.97	2.34
LP	206.31 \pm 61.6	237.54 \pm 62.0	249.85 \pm 61.9	15.14#	21.10\$
Weights					
Upper Body					
CP	105.83 \pm 19.75	110.33 \pm 16.62	115.17 \pm 18.12	4.25	8.83
TA	180.00 \pm 21.22	191.5 \pm 20.02	202.50 \pm 22.61	6.39\$	12.5\$
OP	105.50 \pm 20.42	112.83 \pm 14.91	119.33 \pm 19.21	6.95	13.11\$
SR	160.67 \pm 26.41	181.67 \pm 22.43	194.17 \pm 27.26	13.07#	20.85\$
Lower Body					
LE	142.83 \pm 17.86	155.50 \pm 20.38	163.83 \pm 18.14	8.87\$	14.70\$
LP	211.17 \pm 52.12	231.42 \pm 47.61	252.42 \pm 56.72	9.59\$	19.53#
Weights Plus					
Upper Body					
CP	123.17 \pm 27.47	138.50 \pm 22.82	146.17 \pm 23.84	12.45\$	18.67\$
TA	201.17 \pm 35.86	219.50 \pm 37.61	232.83 \pm 39.73	9.11\$	15.74\$
OP	124.5 \pm 34.00	154.67 \pm 46.28	146.67 \pm 30.60	24.23\$	17.81\$
SR	209.5 \pm 48.24	225.83 \pm 43.37	242.50 \pm 45.10	7.79\$	15.75\$
Lower Body					
LE	165.00 \pm 32.71	188.33 \pm 36.95	196.50 \pm 45.38	14.14@	19.10\$
LP	282.00 \pm 86.64	288.67 \pm 96.24	325.33 \pm 99.54	2.36	15.37\$
Weights Plus+ Drug					
Upper Body					
CP	90.44 \pm 16.36	103.56 \pm 19	123.56 \pm 21.93	14.51@	36.74\$
TA	183.33 \pm 31.23	198.67 \pm 30.51	214.22 \pm 31.06	8.37\$	16.85\$
OP	101.78 \pm 20.06	114.89 \pm 17.24	120.22 \pm 12.31	12.88@	18.12#
SR	167.11 \pm 29.53	191.3 \pm 30.03	201.33 \pm 31.225	14.48\$	20.48\$
Lower Body					
LE	148.44 \pm 27.64	166.89 \pm 33.30	170.22 \pm 36.05	12.43@	14.67@
LP	219.56 \pm 36.25	258.67 \pm 45.92	286.25 \pm 61.676	17.81#	30.37\$

Chest Press

The means and standard deviations for the Chest Press 1-RM are contained in Table 4-7. Absolute changes in 1-RM Chest Press strength are shown in Figure 4-23. Relative changes in Chest Press 1-RM data are shown in Figure 4-24. The only groups to show significant change between T1 and T2 were the Weights Plus (12.45%, $p < 0.001$) and the Weights Plus + Drug (14.51%, $p < 0.05$). Only the Weights Plus (18.67%, $p < 0.001$) and the Weights Plus + Drug (36.74%, $p < 0.001$) groups showing significant change between baseline and T3.

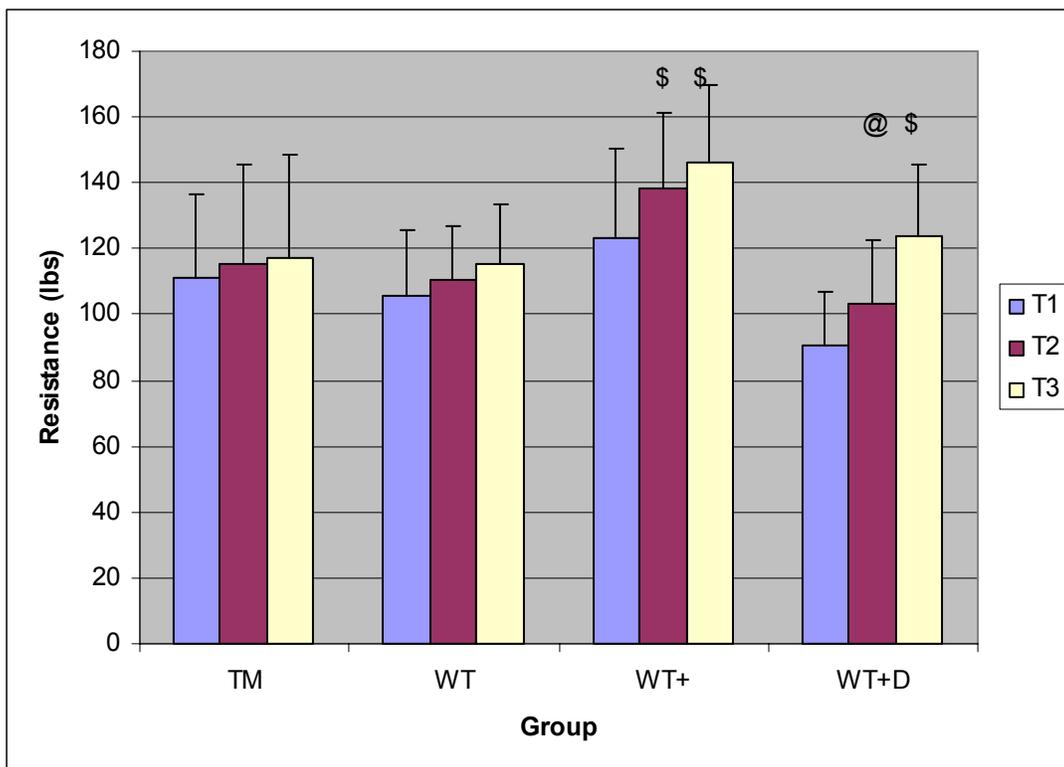


Figure 4-23. Absolute Chest Press 1-RM (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @= $p < 0.05$, \$= $p < 0.001$.

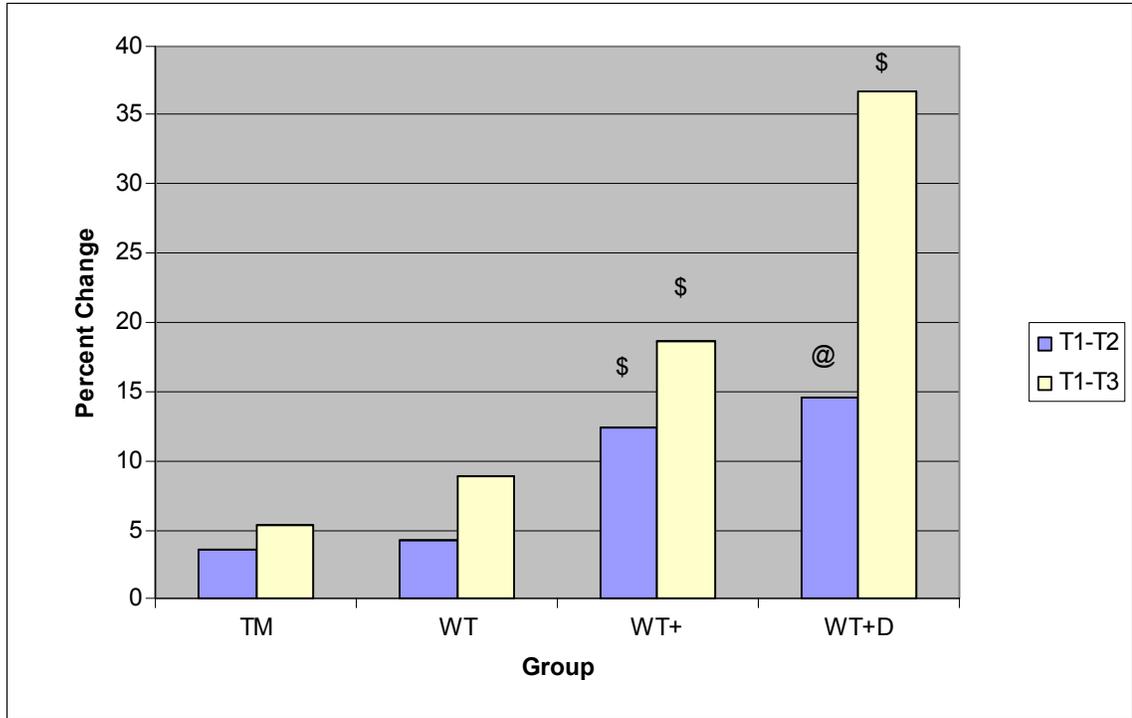


Figure 4-24. Relative Changes in Chest Press 1-Rm. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @= $p<0.05$, \$= $p<0.001$

Torso Arm

The means and standard deviations for the Torso Arm 1-RM test are contained in Table 4-7. Absolute changes in Torso Arm 1-RM are shown in Figure 4-25. Relative changes in Torso Arm 1-RM are shown in Figure 4-26. All groups showed significant improvement between T1 and T2. The Treadmill group had a modest, but significant 4.36% ($p<0.05$) increase in strength. All of the other groups regularly trained on this equipment and also showed significant improvement. The Weights group had a 6.39% increase ($p<0.001$) the Weights Plus group had a 9.11% increase ($p<0.001$) and the Weights Plus +Drug group registered a 8.37% increase ($p<0.001$). Strength increases over baseline continued to T3 for all groups except the Treadmill group. The Treadmill Group which had a significant gain at T2 failed to maintain this increase at T3. Each of

the other groups showed a significant change from T1 to T3. The Weights Group increased by 12.5% over baseline ($p<0.001$), the Weights Plus Group showed a 15.74% increase ($p<0.001$) and the Weights Plus + Drug Group increased by 16.85% ($p<0.001$).

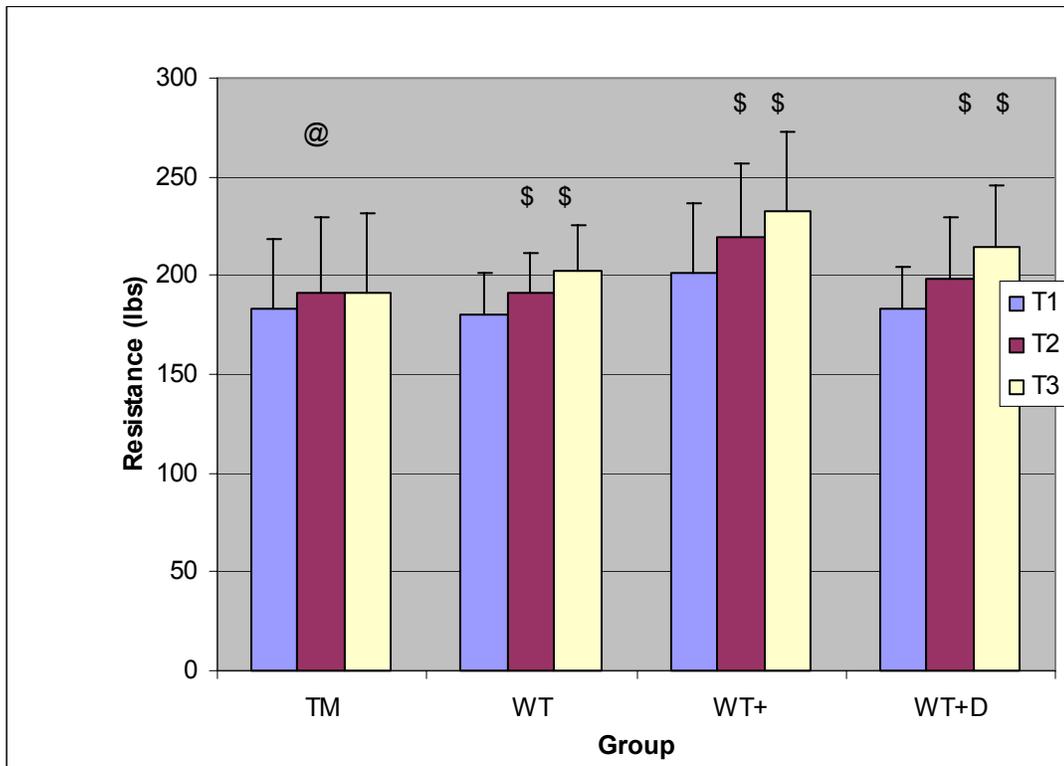


Figure 4-25. Absolute 1RM Torso Arm Strength (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ $p<0.05$, \$ $p<0.001$

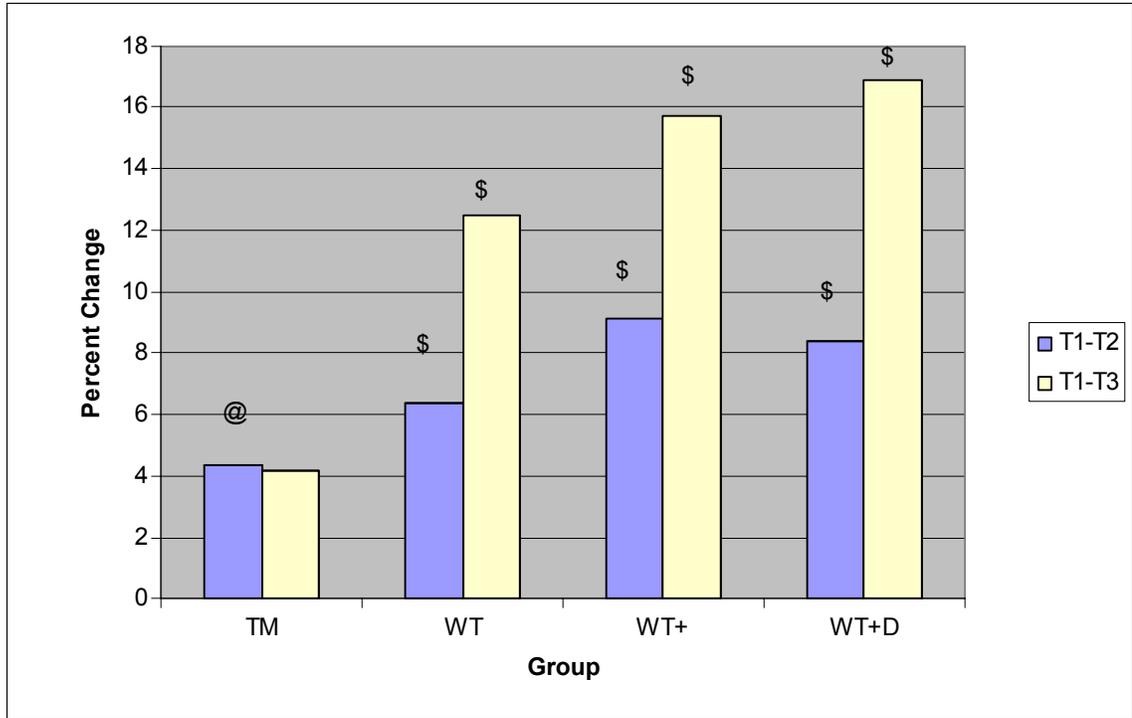


Figure 4-26. Relative Changes in Torso Arm 1-RM. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ $p < 0.05$, \$ $p < 0.001$.

Overhead Press

The means and standard deviations for the Overhead Press 1-RM test are contained in Table 4-7. Absolute changes in Overhead Press 1-RM strength are shown in Figure 4-27. Relative changes in Overhead Press 1-RM are shown in Figure 4-28. The only groups to show significant improvement between T1 and T2 were the Weights Plus (24.23%, $p < 0.001$) and the Weights Plus + Drug (12.88%, $p < 0.05$). By the end of the study the Weights group achieved significant increases over baseline (13.11%, $p < 0.001$). The Weights Plus and Weights Plus + Drug groups also had significant strength gains over baseline (17.81%, $p < 0.001$ and 18.12%, $p < 0.01$ respectively).

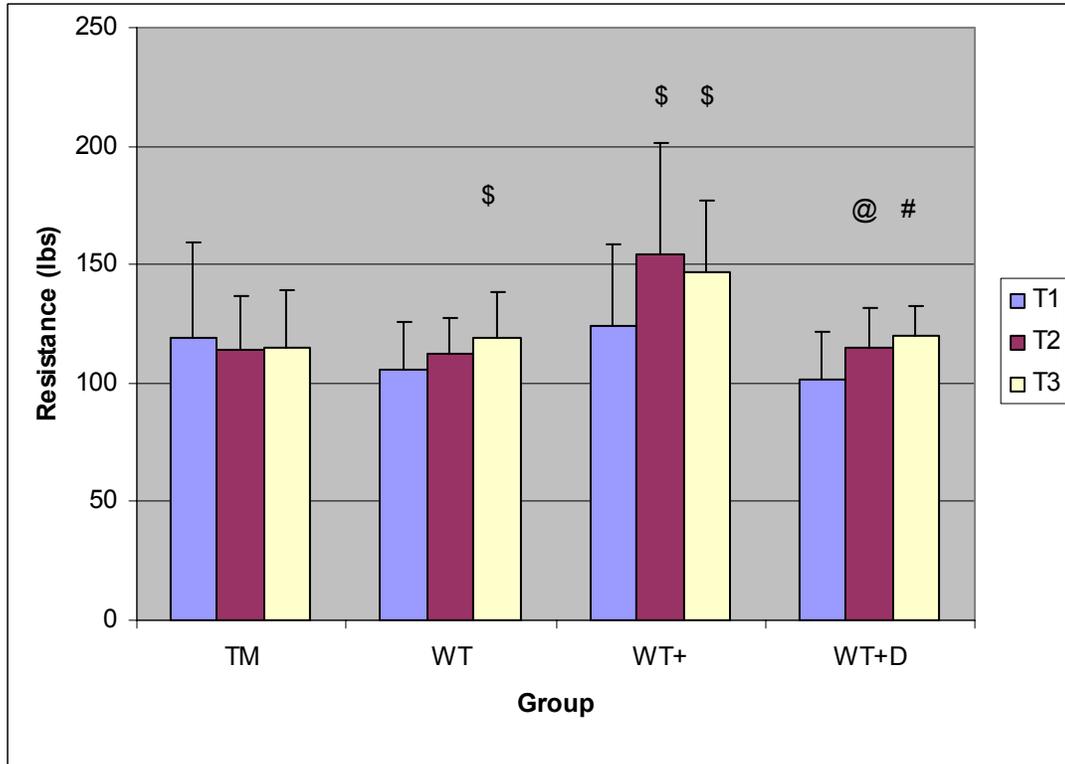


Figure 4-27. Absolute Overhead Press 1-RM (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ p<0.05, # p<0.01, \$ p<0.001.

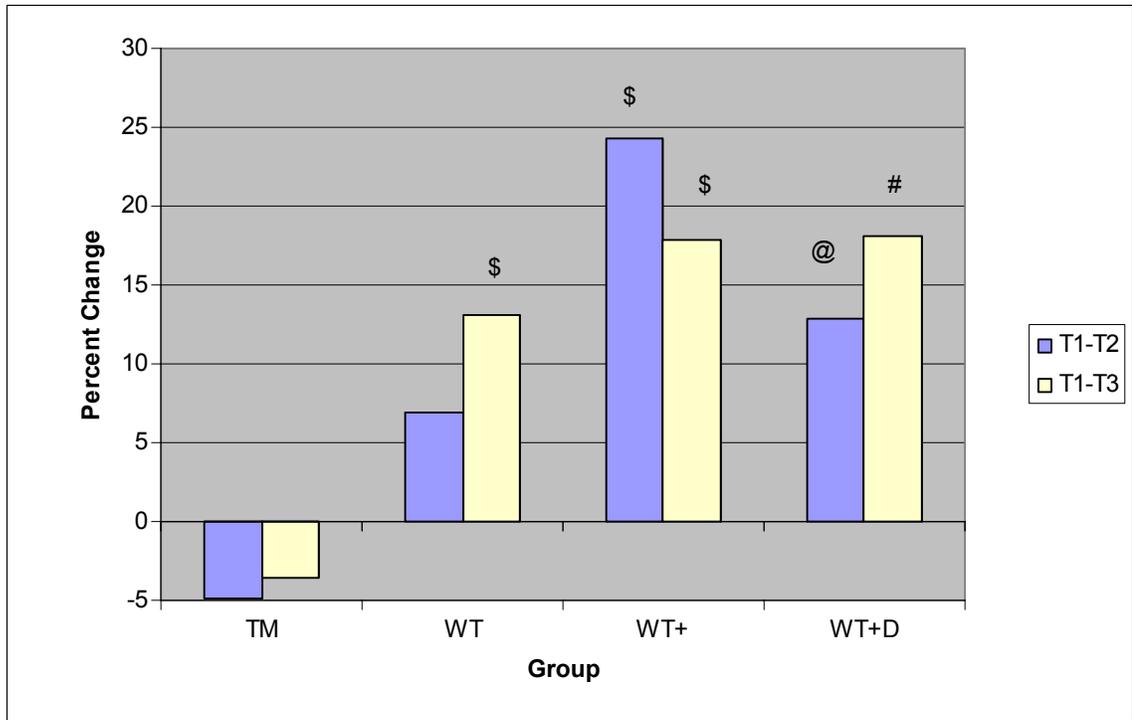


Figure 4-28. Relative Changes in Overhead Press 1-RM. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ $p<0.05$, # $p<0.01$, \$ $p<0.001$

Seated Row

The means and standard deviations for the Seated Row 1-RM test are contained in Table 4-7. Absolute changes in Seated Row 1-RM strength are shown in Figure 4-29. Relative changes in Seated Row 1-RM are shown in Figure 4-30. All groups except Treadmill showed significant improvement at T2. The Weights groups had increases of 13.07% ($p<0.01$). The Weights Plus group increased 7.79% ($p<0.001$). The Weights Plus + Drug group increased 14.48% ($p<0.001$). By the end of the study, however, all groups showed significant improvement over baseline. The Treadmill group achieved a 8.61% increase ($p<0.05$) while the Weights group showed a 20.85% increase ($p<0.001$), the Weights Plus had a 15.75% increase ($p<0.001$), and the Weights Plus + Drug group increased 20.48% ($p<0.001$) at T3.

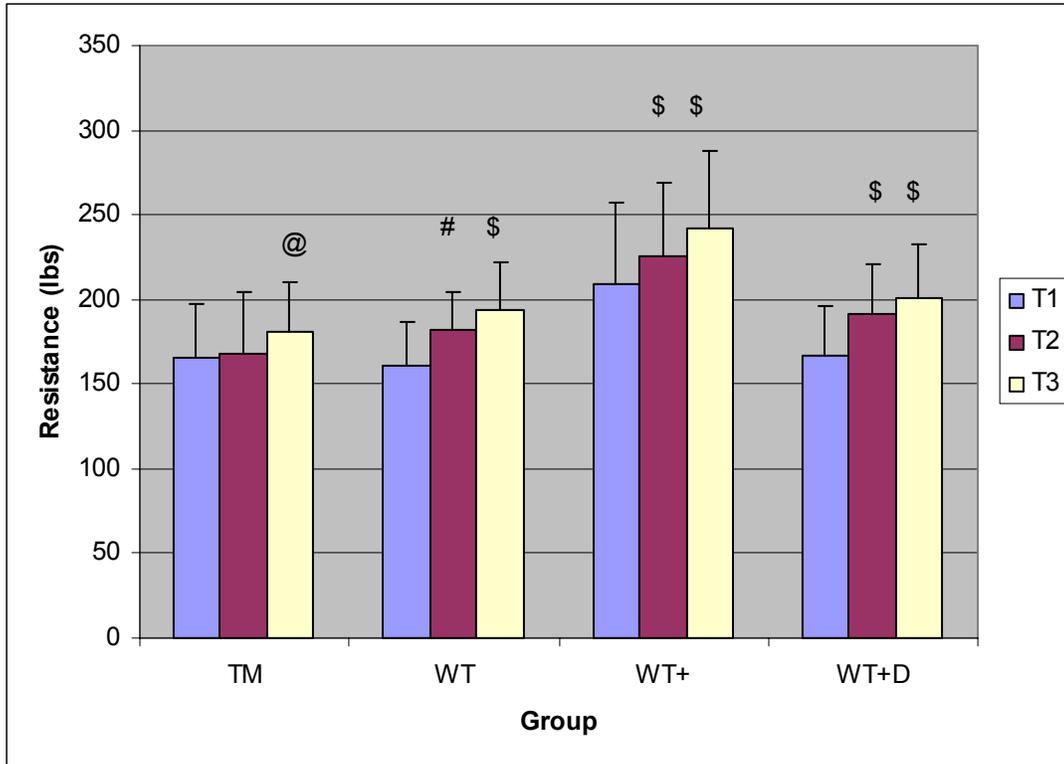


Figure 4-29. Absolute Seated Row 1-RM (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ p<0.05, # p<0.01, \$ p<0.001

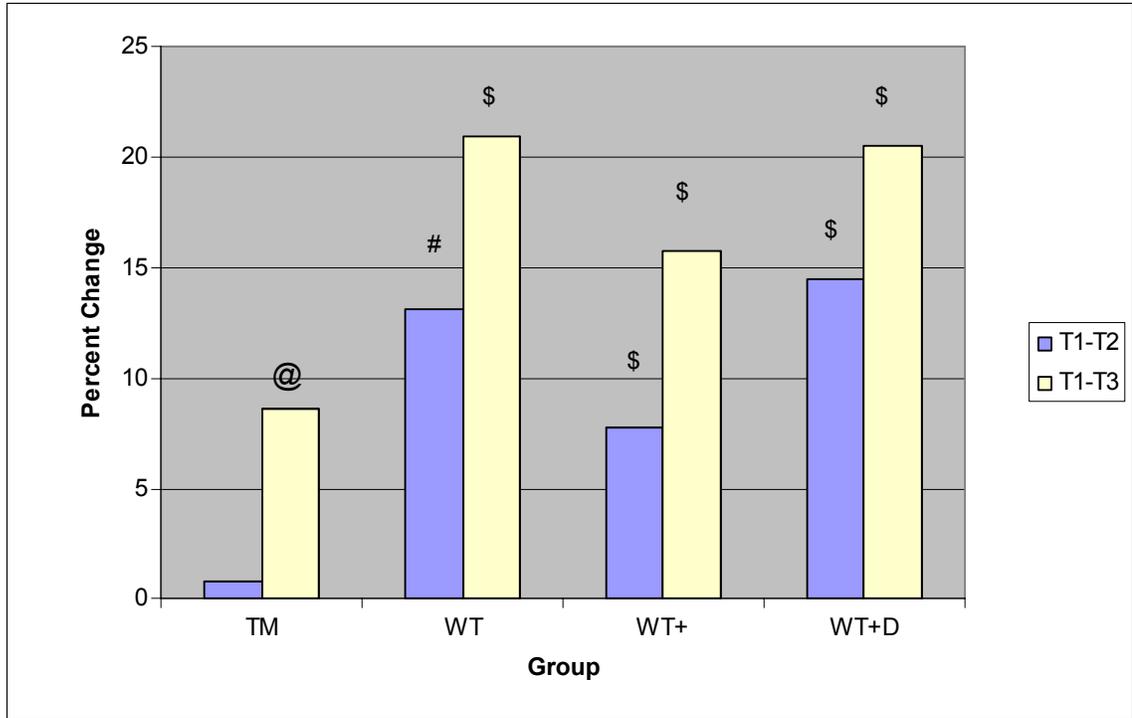


Figure 4-30. Relative Change in Seated Row 1-RM. Values are % change.
 TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group,
 WT+D=Weights Plus and Drug Group. @ $p < 0.05$, # $p < 0.01$, \$ $p < 0.001$

Leg Extension

The means and standard deviations for total the Leg Extension 1-RM test are contained in Table 4-7. Absolute changes in Leg Extension 1-RM strength are shown in Figure 4-31. Relative changes in Leg Extension are shown in Figure 4-32. At T2 all groups except the Treadmill showed significant strength increases. The Weights group had a 8.87% ($p < 0.001$), the Weights Plus Group increased 14.14% ($p < 0.05$), and the Weights Plus + Drug increased 12.43% ($p < 0.01$). The results were similar at T3, with the Treadmill group still not achieving significant strength gains while the other three groups all maintained significant strength gains over baseline. By the end of the study, the Weights Group increased 14.70% ($p < 0.001$), the Weights Plus Group increased 19.10% ($p < 0.001$) and the Weights Plus + Drug increased 14.67% ($p < 0.05$)

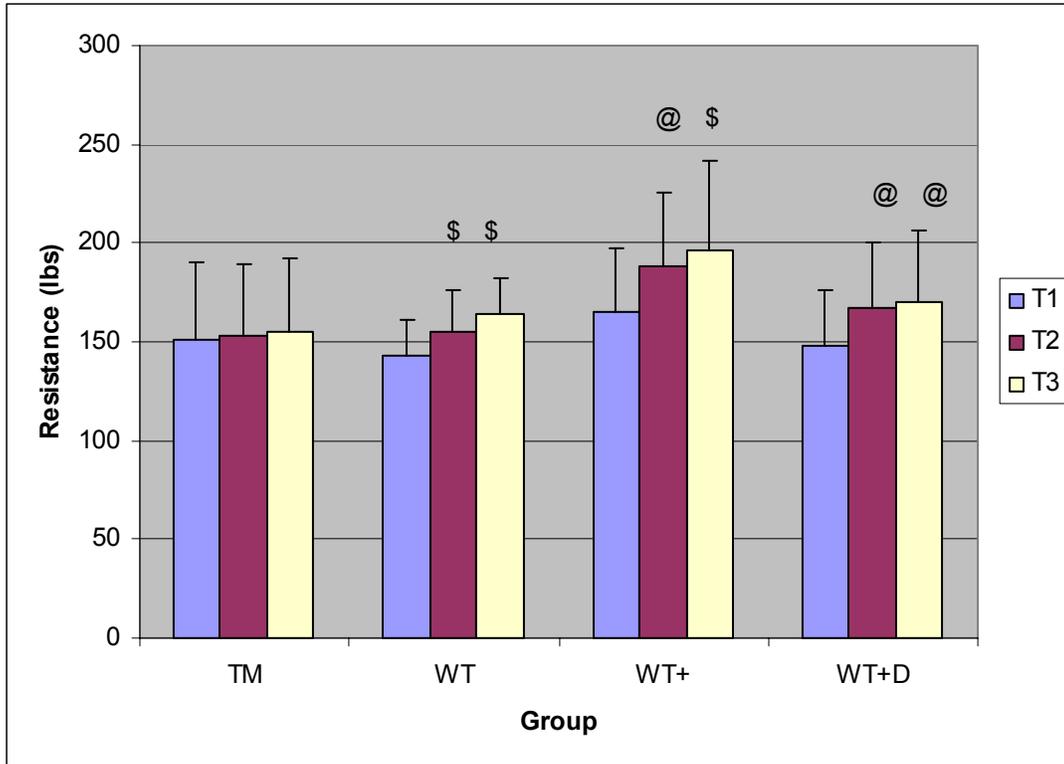


Figure 4-31. Absolute Leg Extension 1-RM (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group.
@ p<0.05, \$ p<0.001

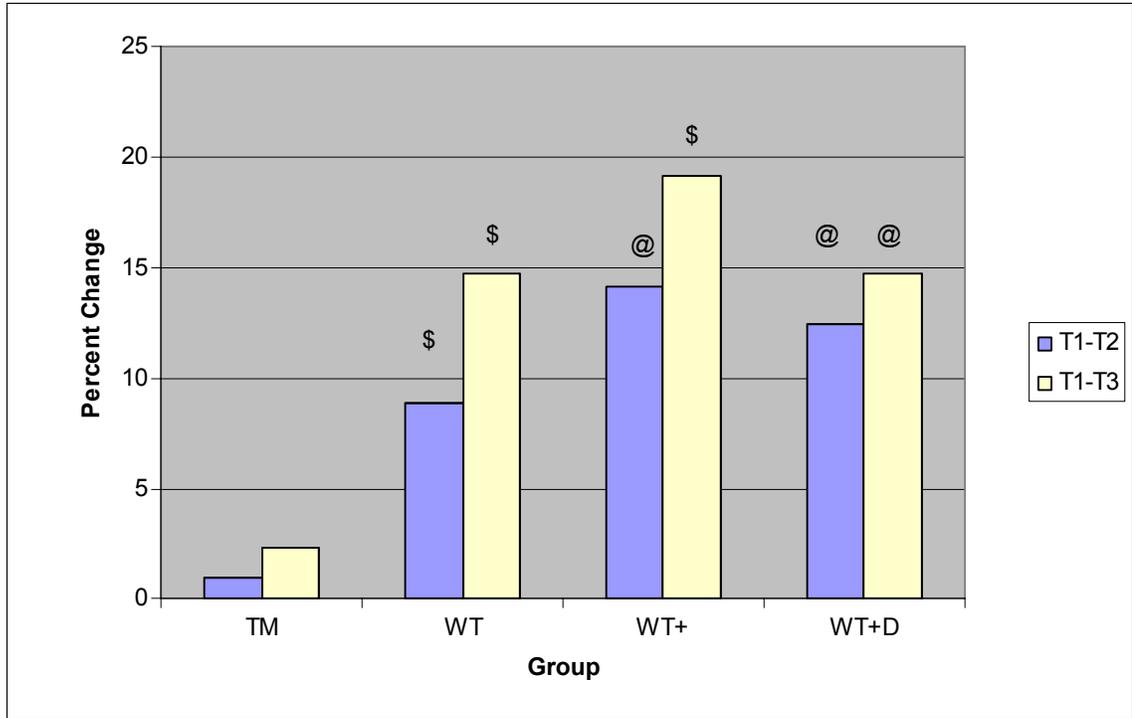


Figure 4-32. Relative Change in Leg Extension 1-RM. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. @ p<0.05, \$ p<0.001

Leg Press

The means and standard deviations for Leg Press 1-RM test are contained in Table 4-7. Absolute changes in Leg Press 1-RM strength are shown in Figure 4-33. Relative changes in Leg Press are shown in Figure 4-34. At T2 all groups except Weights Plus showed improvement. At T2, the Treadmill Group showed a 15.14% (p<0.01) increase. The Weights Group increased 9.59% (p<0.001), while the Weights Plus + Drug group increased 17.81% (p<0.01). By T3, there were increased strength gains over baseline for all groups. The Treadmill Group increased 21.10% (p<0.001), the Weights Group increased 19.53% (p<0.01), the Weights Plus Group increased 15.37% (p<0.001) and the Weights Plus + Drug Group had the highest Relative change with a 30.37% increase (p<0.001).

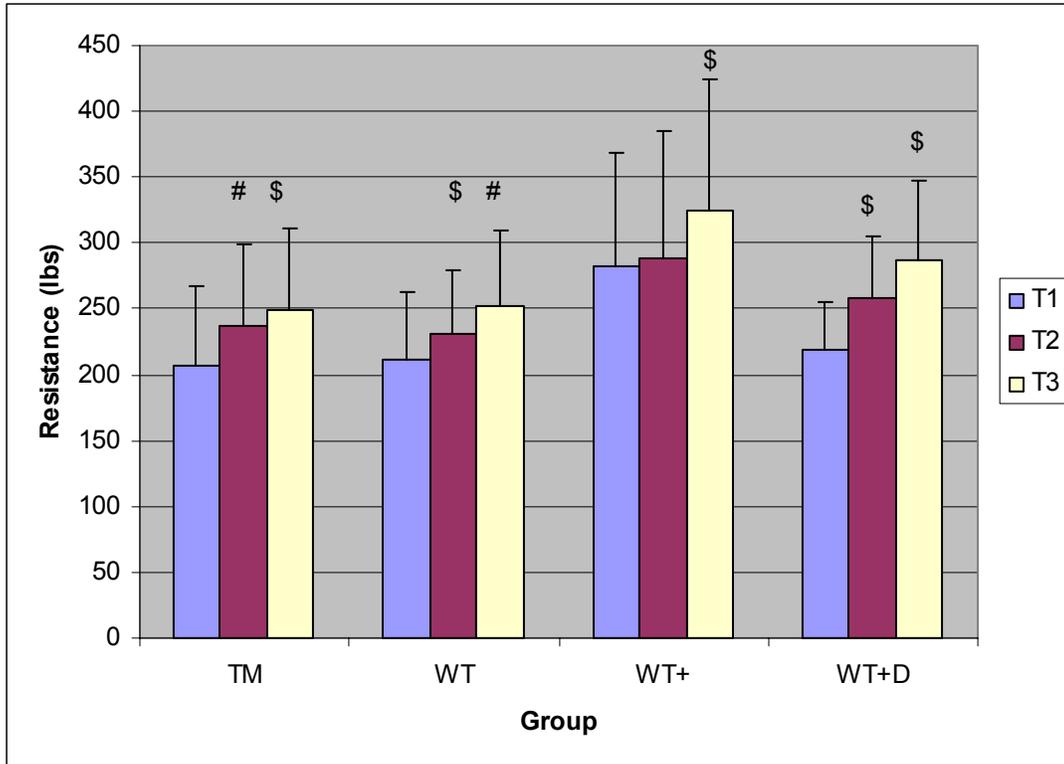


Figure 4-33. Absolute Leg Press 1-RM (lbs). TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. # $p < 0.01$, \$ $p < 0.001$

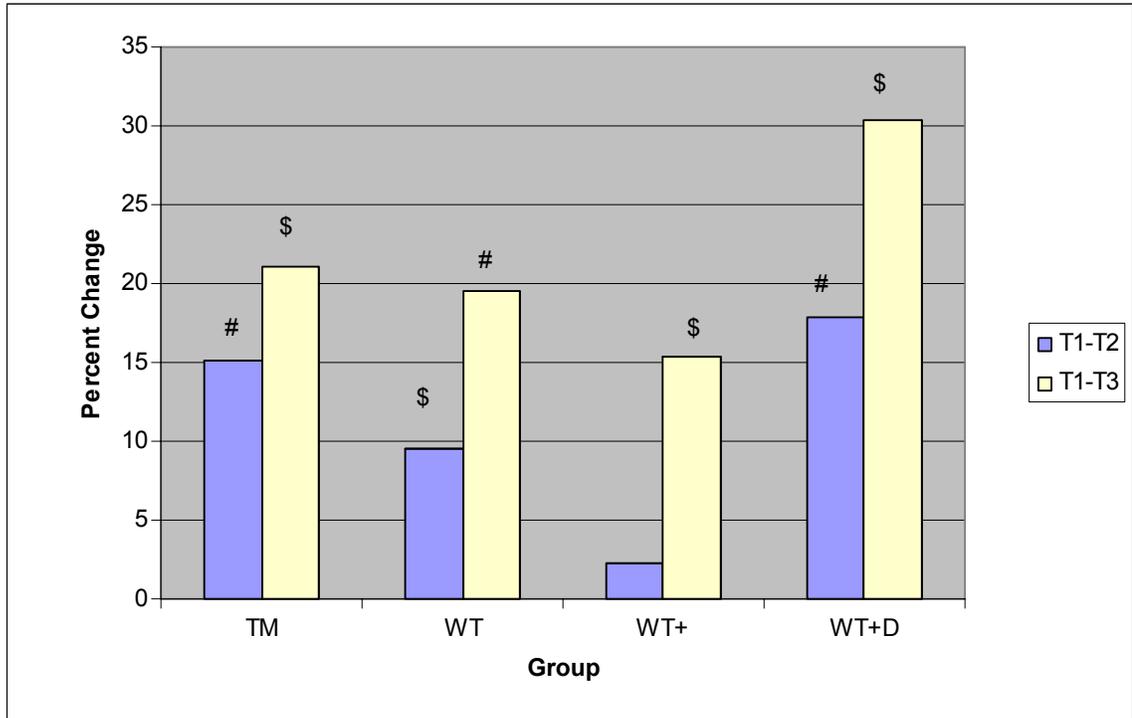


Figure 4-34. Relative Change in Leg Press 1-RM. Values are % change. TM=Treadmill Group, WT=Weights Group, WT+=Weights Plus Group, WT+D=Weights Plus and Drug Group. # $p<0.01$, \$ $p<0.001$

CHAPTER 5 DISCUSSION

Overview and Principle Findings

This study was conducted to determine if an intervention of progressive mechanical loading, utilizing specific clinical and commercial variable resistance exercise machines, is an efficacious method for increasing bone mineral density in healthy postmenopausal women not taking hormone replacement therapy.

When a limb of the Women's Health Initiative study investigating the use of estrogen + progestin was prematurely halted in July 2002, many women chose to halt their estrogen intake and we saw a need to help identify alternative means for postmenopausal women to maintain good bone health (90). With that clinical rationale in mind, we decided to determine the efficacy of a nine-month supervised progressive resistance training program in promoting osteogenesis in this large demographic population at risk for osteoporosis.

The study was designed to test four hypotheses:

1. BMD loss will be attenuated in postmenopausal women not taking hormone replacement therapy following a nine-month, supervised progressive resistance training program.
2. A combination of lower and upper body resistance exercises coupled with the use of the MedX lumbar extension and leg press machines will increase hip and lumbar vertebrae BMD more effectively than either an aerobic exercise program or a resistance training program that excludes the low back extension and leg press machines.
3. A progressive resistance training program will increase serum levels of biomarkers (Osteocalcin and Pyridinoline) indicative of increased bone remodeling and the net change will correlate with increased bone deposition.

4. A supervised nine-month progressive resistance training program will improve skeletal muscular strength in postmenopausal women not taking hormone replacement therapy.

The data support hypothesis #1. Resistance exercise did not significantly increase bone density at any of the measured anatomic regions, but bone losses were below the 1-3% annual losses typically seen in the absence of intervention. This suggests that age-related bone loss was attenuated by mechanical loading. Hypothesis #2 was not supported by the data. There were no significant increases in bone density, so we are unable to conclude that one exercise protocol was more efficacious than the others. The data are mixed with respect to Hypothesis #3. The only significant changes were in pyridinoline crosslinks levels, a marker of bone resorption. PYD increased significantly at T2 in all except the Weights group. However, at T3, significant increases had only significantly increased over baseline in the Treadmill group. Hypothesis #4 was clearly supported by the data. Participants experienced significant strength gains and those involved in resistance training activities achieved a greater level of improvement than did those involved in treadmill walking only.

There have been other exercise studies involving postmenopausal women, but few of these specifically studied women who are not taking HRT. Kerr et al. (38) specifically examined the importance of HRT in bone formation in a resistance training program that included women taking and not taking HRT and reported that the ability to improve bone density using resistance training protocols was significantly better in women receiving HRT. Their data supports our finding that women not receiving HRT have a particularly difficult time increasing bone density due to their lack of estrogen, even with a loading regimen known to be osteogenic in other populations at risk for osteoporosis.

While we did not observe significant gains in BMD, our findings provide support for the idea that progressive resistance training can attenuate age-related declines in BMD. Bone loss is common in women following menopause. In the first five to seven years following menopause, BMD declines an average of 3% per year. After that, further bone loss plateaus at about 1% per year (17, 61). From the data collected in this study it appears that resistance training alone may not be a sufficient stimulus to increase bone mineral density in postmenopausal women not taking HRT. For these women, a resistance training program results in significant strength gains but not significant increases BMD. If these women cannot maintain appropriate levels of BMD, pharmacologic intervention may be necessary.

Bone Mineral Density

The exercise protocol used in this study did not significantly increase BMD. This suggests it is particularly difficult for women not taking HRT to maintain or gain BMD. The only significant changes in bone density during the study were losses in Total bone BMD in the Weights Plus + Drug group at T3 and at the Femur neck in the Weights Group at T2. These findings, however, do not tell the whole story. It is well documented that women begin experiencing increased bone loss following menopause (61). These losses are greatest in the first 3-5 years of menopause when they can approach 3% per year. After that, losses tend to moderate to an average of about 1% per year. Therefore, in the absence of any intervention we would have expected the study subjects to lose almost 1-3% of their bone mass during the study. In contrast, participants in this study did not lose significant BMD between study entry and study conclusion (about 10 months).

Total Body Bone Mineral Density

A small decrease in total body BMD was observed in this experiment and mirrors similar findings in other studies. In this study, total body BMD decreased only in the Weights Plus + Drug group. This loss was significant at both T2 (-0.952%, $p < 0.01$) and T3 (-0.667%, $p < 0.05$) (Figure 4-1). At study entry, this group was already receiving anti-osteoporotic drugs and may have been more prone to further bone loss.

Lohman et al. (43) found losses in total body BMD following an 18-month resistance training program in pre-menopausal women. Menkes et al. (52) also found similar Total body BMD losses in middle-aged and older men. Menkes (52) speculated that since bone accretion is site specific, the loss of total body BMD might reflect a redistribution of bone. While we also observed a loss of total body BMD, our data did not support a theory espousing a redistribution of existing bone. Rather, we believe the Total body BMD losses may reflect the fact that mineralization lags behind bone formation. Bone is lost primarily from the endosteal surface, while bone accretion takes place at the periosteum. This means that bones are constantly increasing in diameter. Since bone density is a measure of the amount of hydroxyapatite present in the total volume of bone, an increase in size would lessen overall bone density until mineralization of new osteoid occurs.

Femur Neck Bone Mineral Density

The data suggest that Femur neck BMD did not significantly change during the study except for a statistically significant loss (-2.133%, $p < 0.05$) in the Weights group at T2. From the data it appears that the groups not targeting the femur neck region with specific exercises showed the greatest losses (Figure 4-3). No decline in femur neck BMD was observed in groups performing the leg press exercise.

We hoped to increase femur neck BMD by targeting this region with the leg press machine. When there is a hip fracture, the site of that fracture is the femur neck 90% of the time. For this reason, we identified the femur neck as a region of interest in this study. In all of the resistance training groups femur neck BMD at the end of the study was similar to that measured at study entry, while the Treadmill group had experienced about a 2% loss in that region. This suggests that the resistance training program was successful in attenuating the loss of BMD at the femur neck, but did not result in increased BMD at this site.

Other, longer studies have shown small improvements in BMD at hip sites. One such study, conducted by Nelson et al. (57) found a modest $0.9\% \pm 4.5\%$ gain of bone following a one-year intervention in postmenopausal women not taking HRT. These results, like ours, show that there is fairly high variability in this group of subjects. Kerr et al. (38) in a one-year resistance training study reported no increase at the femur neck ($0.0\% \pm 3.1$) but reported slight gains at the trochanter.

Difficulties in increasing femoral neck BMD have been previously documented by researchers. Bassey and Rothwell (1) conducted a study on pre-and postmenopausal women to determine the effects of jumping on femoral BMD. They reported significant changes in premenopausal women after six months, but did not get results in postmenopausal subjects even after 1 year of intervention. Additionally, Cussler et al. (22), in a recently published study, found that femur neck and lumbar spine BMD changes were not related significantly to total weight lifted or to exercise-specific activities. These varied findings by other researchers support our finding that it is

difficult for postmenopausal women to increase BMD in this anatomic region with resistance exercise alone.

Trochanter Bone Mineral Density

The study protocol was not effective in stimulating mineralization of the trochanter in any group, although by the end of the study the groups which specifically targeted the hip region showed modest, but not significant, increases in trochanter BMD. Other studies have found modest trochanter BMD changes after 1 year of intervention. Kerr et al. (38) conducted a one year progressive exercise program in postmenopausal women (natural and surgical) not taking HRT and reported modest significant bone density change ($1.7\% \pm 4.1$) only in the trochanter. Cussler et al. (22) in a one-year free weight resistance training program for women in early postmenopause only achieved modest significant results in the trochanter. They speculated that contractile forces associated with the exercise protocol had more impact on the trochanter than other hip sites like the femur neck because of the location of the attachments of targeted muscle groups.

Lumbar Spine Bone Mineral Density

In postmenopausal women, loss of bone is often greatest and most easily detected in the lumbar spine. This is because the vertebrae have a high percentage of trabecular bone, the type of bone most susceptible to remodeling.

Our data show no significant change in lumbar spine BMD as a result of the exercise interventions. However, the data suggests that resistance training attenuated age-related losses that would have been expected in the absence of an intervention.

Other studies have shown mixed results at the lumbar spine. Maddalozzo and Snow (45) conducted a 24-week strength training program in men and women ages 50-60. They found lumbar spine bone adaptations to the exercise program in men but not in

the women. On the other hand, Nelson et al. (57) reported a very small but significant ($1.0\% \pm 3.6$) gain in lumbar spine bone density following a twelve-month resistance training program in women not taking HRT. Some researchers, however, have even reported difficulty in increasing BMD in the lumbar spine of premenopausal women. Sinaki et al. (77) reported no change in lumbar spine bone density even after a three-year resistance training intervention which used an intensity level of 3 sets of 10-RM weights three days per week.

Serum Markers

Osteocalcin

Osteocalcin is a marker of bone formation. It is secreted by osteoblasts in the late stages of their differentiation and is found exclusively in bone tissue (74). Serum osteocalcin levels were measured in subjects at study entry, T2 and T3. Serum osteocalcin levels are typically around 3.7-10 ng/ml. Seiffert-Krauss et al. (74) found that osteocalcin levels tend to increase slightly in postmenopausal women. This may occur because osteoblast and osteoclast activity are related and osteoclastic activity is elevated in postmenopausal women (74). In this study, serum osteocalcin levels did not change significantly for any group during this study. We would expect osteocalcin levels to increase if there were increased bone formation.

Pyridinoline

Serum pyridinoline levels were measured in study participants. Pyridinoline crosslinks (PYD) are a marker of bone resorption. Since bone resorption precedes formation, we expected PYD levels to increase. At T2, PYD levels had significantly increased in every group except Weights Plus. The Treadmill group had a 11.38% ($p < 0.05$) increase, the Weights group increased 15.63% ($p < 0.05$), and the Weights Plus +

Drug group increased 11.92% ($p < 0.05$). At T3 all groups except Weights Plus had increased over the baseline, but the only significant increase was seen in the Treadmill group. Seiffert-Krauss (74) in a study comparing the levels of various biochemical markers in peri- and postmenopausal women found that there are higher levels of pyridinoline crosslinks in postmenopausal women. This is consistent with the fact that postmenopausal women have a higher rate of bone turnover and that pyridinoline crosslinks reflect this.

Body Composition

The exercise programs used in this study did not significantly alter body composition. The study was not designed to be a weight loss program. The groups exercised twice weekly for 30-40 minutes. Other, studies with similar designs have also found, at best, only modest changes to body composition in this age group and gender. Following a one-year resistance training program, Teixeira et al. (79) found that women not on HRT gained about 1 kg in lean tissue. Our findings reflect a similar trend, but did not reach statistical significance. The difference in results may reflect the fact that Teixeira's study was longer than ours. In terms of lost fat tissue, Teixeira reports an average fat mass loss of 0.4 kg. Although we recorded losses, they did not achieve levels sufficient to be significant.

Strength

The exercise protocols were effective in increasing strength, particularly among those groups engaged in resistance training. Strength assessments are important in bone research because of the interaction of muscle and skeletal components. There is evidence that strength training benefits individuals as they grow older. A lack of muscle tone can affect balance and increase the risk of falling (4). Frost (27) also has suggested that age-

related sarcopenia is related to bone loss. Sinaki et al. (76) also suggests that a difference exists in muscle strength between women with and without osteoporosis, and that muscle mass provides an independent predictor of who is at risk for age-related bone loss (75). It is estimated that the age-related loss of lean muscle tissue is about 1% per year beginning in at about the age of 40 (3). Other studies suggest that women experience a 15% loss in muscular strength during the years around menopause (4). The benefits of resistance training include increased muscle and bone mass, muscle strength, self-confidence and self-esteem (73).

Previous studies indicate the efficacy of strength training for increasing muscle strength in postmenopausal women. Nelson et al. (57) found a 35-76% increase in strength in 50-70 year old women following a one year progressive exercise program. In this study Nelson, using creatine excretion to estimate total body muscle mass, reported a 1.2 kg increase. A 12 week resistance training program conducted by Skelton et al. (78) found a smaller but significant 4-27% increase in women over the age of 75. These strength data are similar to our findings.

Lumbar Extensor Strength

Increases in back strength are important to reducing the risk of vertebral fracture. Sinaki (75) found that female patients with osteoporotic fractures had weaker back extensor muscles compared to patients without such fractures. In the present study, isometric lumbar extensor strength (measured by the MedX Strength Index) increased in all resistance trained groups, but did not increase in the Treadmill group. The Weights group, which did no exercises specifically targeting these muscles, achieved a significant gain in strength only by the end of the study (20.86%, $p < 0.05$). In contrast, both groups that specifically trained these muscles had significant improvement both at the T2 and

T3. At T2, the Weights Plus group demonstrated a 38.98% ($p < 0.01$) increase, while the Weights Plus + Drug group posted gains of 29.56% ($p < 0.01$). At T3, The Weights Plus group increased their lumbar strength by 51.14% ($p < 0.01$) above baseline while the Weights Plus + Drug group increased their strength by 33.85% ($p < 0.01$) over baseline (Table 4-6). Our findings with respect to lumbar extensor strength gains support previous studies.

Pollock et al. (66) in a study with young men and women (mean age 29.1 ± 8 years), using similar equipment once per week for 10 weeks reported gains of 42% at full flexion (72^0) and 102% at full extension (0^0). As with previous studies, we found that the most significant strength gains came in the position of full extension (0^0). The Weights Plus group had an overall 86.73% gain ($p < 0.01$) and Weights Plus + Drug groups showed a 108.2% gain ($p < 0.01$) in isometric strength at this angle. Carpenter et al. (14) also found that most of the strength gain among young adults using this equipment are achieved primarily in the first 12 weeks, with smaller gains realized in later weeks. Strength gains in this group of healthy young men and women ranged from 16.4% at full flexion (72^0) to 91.9% at full extension (0^0). We found similar patterns of strength gains in groups using this equipment with most of the gains coming in the first half (4.5 months) of the study.

1-RM Strength

1-RM strength testing was conducted on each of the 46 women in the study at baseline and after 4.5 and 9 months of exercise intervention. 1-RM testing was conducted using MedX Chest Press, Torso Arm, Overhead Press, Seated Row, Leg Extension, and Leg Press machines. The results show that the resistance training program used in this study resulted in significant strength gains. The Treadmill group made significant gains (4.36%, $p < 0.05$) only at T2 in the Torso Arm exercise. The

Weights group made significant gains by T2 in the Torso Arm (6.39%, $p<0.001$) Seated Row (13.07%, $p<0.01$), the Leg Extension (8.87%, $p<0.001$), and the Leg Press (9.59%, $p<0.001$). The Weights group continued to make improvement and by T3 had made significant strength gains over baseline on the Torso Arm (12.50%, $p<0.001$), Overhead Press (13.11%, $p<0.001$), Seated Row (20.85%, $p<0.001$), Leg Extension (14.7%, $p<0.001$), and the Leg Press machines (19.53%, $p<0.01$). The Weights Plus group made significant gains in all exercises except the Leg Press at T2 and in all exercises by T3. At T2, strength gains for the Weights Plus group included: Chest Press (12.45%, $p<0.001$), Torso Arm (9.11%, $p<0.001$), Overhead Press (24.23%, $p<0.001$), Seated Row (7.79%, $p<0.001$), and Leg Extension (14.14%, $p<0.05$). By T3, the Weights Plus group registered significant strength gains over baseline in the Chest Press (18.67%, $p<0.001$), Torso Arm (15.74%, $p<0.001$), Overhead Press (17.81%, $p<0.001$), Seated Row 15.75%, $p<0.001$), Leg Extension (19.10%, $p<0.001$) and the Leg Press (15.37%, $p<0.001$). The Weights Plus + Drug group showed significant improvement in all exercises at both T2 and T3. At T2, changes for this group include Chest Press (14.51%, $p<0.05$), Torso Arm (8.37%, $p<0.001$), Overhead Press (12.88%, $p<0.05$), Seated Row (14.48%, $p<0.001$), Leg Extension 12.43%, $p<0.05$) and the Leg Press (17.81%, $p<0.01$). At T3 the Weights Plus + Drug group's gains over baseline were even greater: Chest Press (36.74%, $p<0.001$), Torso Arm (16.85%, $p<0.001$), Overhead Press (18.12%, $p<0.01$), Seated Row 20.48%, $p<0.001$), Leg Extension (14.67%, $p<0.05$), and the Leg Press (30.37%, $p<0.001$).

These findings are similar to those in other resistance training programs. Nelson et al. (57) reported strength gains ranging from 35-76% following a twelve-month

resistance training designed stimulate bone accretion. Kerr et al. (38) also reported significant changes in strength during a one-year study using free-weights..

Limitations to the Experiment

This experiment was designed to test the efficacy of various exercise protocols on a segment of the population which has particular difficulties adding BMD to either the axial or appendicular skeleton. Several other studies investigating the efficacy of exercise in postmenopausal women have lasted 12 months or longer (38, 43, 77), but even with the extended length of intervention, gains in BMD were modest. However, clinical populations with rapid-turnover osteoporosis/osteopenia appear to respond more robustly to resistance training interventions that are less than one year in duration. Several related studies (7-12, 53, 54, 87) involving organ transplant recipients with glucocorticoid-induced osteoporosis report significant BMD gains in a short period of time, but studies on generally healthy populations may require a longer intervention. This appears to be the case, especially with postmenopausal women not taking HRT. Studies which have included women both on and not on HRT showed that those on HRT fare significantly better in building BMD in response to resistance exercise (22, 79). In this study, we were able to successfully attenuate bone loss but were not able to significantly increase BMD at any of the measured sites in our study subjects.

The study protocol was designed to implement a realistic exercise regimen that would be accessible to the general population and could be adopted as a lifestyle strategy to combat bone loss. What we found, however, was that many women in this study were unable to complete an uninterrupted progression in weights. Several reported generalized joint soreness at about the 4-5 month point of the study. To insure their safety, weights were lowered or exercises halted until their discomfort subsided. Once they were again

pain-free, weights were gradually increased. This prevented us from maintaining a constant progression of weights and may have prevented subjects from maintaining sufficient overload stress essential for stimulating bone remodeling. Other studies have not reported the musculoskeletal symptoms in their subjects, so there is no way to determine how common this phenomenon may be.

The length of time that our subjects were menopausal may also have influenced results. Ohta (61), among others, has traced the rate of bone loss in women in the late premenopausal to postmenopausal years. Bone loss begins to increase at a higher rate during perimenopause and accelerates during the first years of menopause. Many researchers believe increased acceleration of bone loss occurs for 5-7 years following menopause, and then moderates to a rate of about 1% per year. Given that 18 of our 46 subjects were under the age of 56, and 31 of the 46 were under the age of 60, we may have had a significant portion of the subjects still experiencing accelerated annual bone losses in excess of 1% per year. This accelerated level of bone loss would have been difficult to overcome in a nine-month study employing only mechanical loading.

Additionally, we also recruited subjects from the local area through newspaper advertisements. This automatically creates the potential for bias because applicants tended to be 1) sensitized to the problems associated with bone loss and 2) willing to exercise. For example, the women in this study averaged 103.9% of the age-matched total body BMD. Femur neck BMD was 95.89%, trochanter BMD was 100.64% and lumbar spine as 102.86% of the age-match expected values. We may have introduced selection bias by recruiting more heavily from a segment of the population which ,

because they were already slightly above the expected BMD (except at the Femur neck), were less apt to show improvement.

Our criteria for selection was rigorous. We were looking for generally healthy women not currently engaged in exercise programs. We only accepted about 35-40% of the women who applied. The stringent acceptance criteria might have resulted in women who were a little more active in their daily lives and may have been too healthy to realize some of the adaptations we were looking for during a short-duration study.

Future Experimental Considerations

When designing studies for this demographic group, it is important not to assume that all postmenopausal women are the same. For example, it is important to distinguish between natural and surgical menopause. Ohta et al. (61) argues that a woman who experiences surgical menopause will experience postmenopausal bone loss at a rate greater than women experiencing natural menopause. Therefore, not mixing natural and surgical menopause subjects helps keep the subject pool more homogeneous.

We found that supervision of exercise is an important key in designing a study. With few exceptions we had a constant ratio of one trainer to one subject and this allowed close supervision of the protocol. Other studies have had ratios of several subject per trainer or have had subjects self-report their activities. While our level of supervision was difficult to maintain, it improves safety and allows researchers to ensure that proper exercise form is maintained.

Future experiments of this nature could attempt to control for some of additional variables that we were unable to control for. First, we may not have adequately taken into account the stages of bone loss that women go through after menopause. Any future study should use block randomization to distinguish between those in the first decade

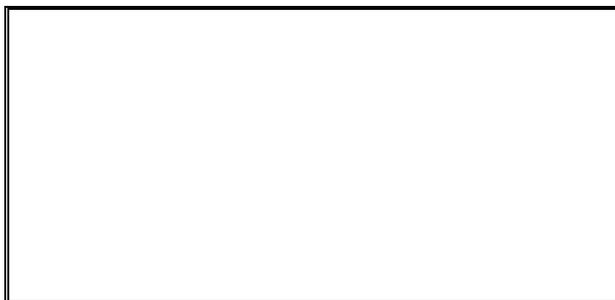
after menopause and those who are beyond that point. Second, a study such as this should have a minimum length of one year for a healthy population with no underlying medical conditions that affect bone metabolism. Third, future studies should use an Anterior-Posterior (AP) spine DXA scan for measurement rather than the lateral scan that was used in this study. A lateral scan typically only allows two vertebrae to be used for measurement and three vertebrae is likely the minimum necessary to achieve consistent and accurate results. Fourth, the study only examined the femur neck and trochanter. Newer techniques and equipment allow for additional analysis including total hip, hip access length, and differential measurements of portions of the femoral neck. These additional measurements would more accurately measure subtle changes in BMD. I would also conduct two scans at each measurement point and average the results. If the two scans differed by more than 5%, a third scan would be performed. Lastly, we need to move beyond reliance on BMD as a measure of adaptation to resistance training interventions. BMD is an important measure and it is the one most familiar to clinicians and the population at large, but BMD does not tell the whole story. Studies with bisphosphonates show that even small increases or decreases in bone density have much greater effects on fracture risk (70). This is because the quality of the bone and the location of bone deposition is just as important, if not more important, than the quantity. DXA techniques cannot measure bone quality but recent advances such in Computerized Axial Tomography (CAT) and Magnetic Resonance Imaging (MRI) allow us to look at the bone microarchitecture in ways never before possible. Since these techniques are non-invasive and non-destructive they offer unprecedented opportunities for serial measurements of the same bone tissue. Future research should incorporate these

techniques. We may find that bone strength changes (and fracture risk decreases) even in the absence of detectable changes in bone density.

APPENDIX A
INFORMED CONSENT

IRB# 458-2002

***Informed Consent to Participate in Research
and Authorization for Collection, Use, and
Disclosure of Protected Health Information***



You are being asked to take part in a research study. This form provides you with information about the study and seeks your authorization for the collection, use and disclosure of your protected health information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. Your participation is entirely voluntary.

1. Name of Participant. (“Study Subject”)

2. Title of Research Study

Resistance Exercise as Therapy for Postmenopausal Osteoporosis

3. Principal Investigator and Telephone Number(s)

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4. Source of Funding or Other Material Support

University of Florida

5. What is the purpose of this research study?

In the United States, 50% of women suffer bone fractures after the onset of menopause. Of the more than 1.5 million fractures that occur each year in postmenopausal women, over 300,000 are fractures of the hip and 700,000 involve the spinal vertebra. The hip and vertebral fractures are caused by low bone thickness. No effective drug therapy exists to increase bone thickness in postmenopausal women. The purpose of this study is to determine the effects of different exercise programs on bone thickness. We believe that specific exercise programs will slow the loss of bone and, perhaps, even result in increased bone formation. Sixty healthy postmenopausal women will be included in the study. You are being asked to participate because you have experienced natural (non-surgical) menopause for at least one year and have not received hormone replacement therapy for at least six months.

6. What will be done if you take part in this research study?

Participant Screening and Group Assignments: Prior to acceptance into the study, you will fill out a health questionnaire to determine your eligibility for the study. If you are accepted into the study, you will report to the Center for Exercise Science in the Florida Gymnasium at the University of Florida or to Medical Exercise Associates in Daytona Beach, Florida for a test that will measure your current bone thickness. At that time a 20 ml (two tablespoons) sample of blood will also be drawn from a vein in your forearm. You will also be given instructions on how to fill out a diet analysis worksheet so that we can determine your average daily intake of calcium. This visit will last approximately 1 hour.

You will then be assigned to one of three 9-month exercise programs: Group #1 will perform 30-40 minutes of treadmill walking exercise 2 days per week at 65-80% of peak heart rate. Group #2 will perform weight-lifting exercises 2 days per week using 11 machines, including the clinical low-back machine and the leg press machine. Group #3 will perform weight-lifting exercises 2 days per week using 9 machines and will not include the clinical low-back machine and the leg press machine.

Bone Thickness Testing: Individuals accepted into the study will report to the Center for Exercise Science at the University of Florida or to Medical Exercise Associates in Daytona Beach, Florida where they will undergo measurement of whole body and

regional bone thickness. Participants will lie on their back or on their side while the X-ray scanner performs a series of scans. This procedure will be performed under the supervision of a licensed X-ray technician and takes approximately 40 minutes. This test will be repeated after 4.5 and 9 months of exercise training to determine the effects of the exercise protocols on bone thickness. Participants will also be offered the opportunity for additional annual bone scans for a period of five years after the exercise program has ended. This will be done to document the long-term effects of the exercise program.

Strength Testing: Prior to starting your 9-month exercise program, skeletal muscle strength will be measured in all study participants. This testing will be done at the Center for Exercise Science in the Florida Gym at the University of Florida or at Medical Exercise Associates in Daytona Beach, Florida. Muscular strength of your lower back will be measured on the MedX low-back machine at seven different positions (0°, 12°, 24°, 36°, 48°, 60°, 72° of low-back flexion). After completion of the low-back strength tests, your arm, leg, chest, shoulder and upper-back strength will also be determined by measuring the maximum amount of weight that you can lift one time. Arm and chest strength will be determined on a MedX chest press machine. During this test the arms are extended away from the chest while pressing against the maximum weight that can be resisted. Leg strength will be determined on a MedX knee extension machine and leg press machine. During this test you are seated and the lower leg is fully extended while lifting the maximum weight that can be resisted. Upper-back strength will be determined on a MedX pull down machine and seated row machine. During these tests you will pull the maximum weight you can. Shoulder strength will be determined using a MedX shoulder press machine. During this test you will push the maximum weight possible over your head. Strength testing visits will last approximately 2 hours. All strength tests will be repeated after 4 ½ and 9 months of the exercise programs (weight training and treadmill walking groups).

Treadmill Walking Program: The treadmill walking program will be available in 2 locations: (1) at the Center For Exercise Science in the Florida Gym at the University of Florida or (2) at Medical Exercise Associates, Daytona Beach, Florida. You will do all of your training and testing at the facility where you enter the study (either Gainesville or Daytona Beach). You will be asked to walk twice weekly on a treadmill for 30-40 minutes at a speed and elevation that causes the heart to beat at 65-80% of age-predicted peak heart rate. Peak heart rate will be estimated by subtracting your age from 220. During all treadmill sessions, your heart rate will be continuously monitored using a Polar heart rate monitor. You will begin the treadmill program gradually by walking for 20 minutes at 50% of estimated peak heart rate. Walking duration will increase by 5 minutes each week until you are walking continuously for 40 minutes. Treadmill speed and/or elevation will be gradually increased until you are walking at 70-80% of your estimated peak heart rate after 8 weeks. Treadmill speed will be capped at 3 mph, however, to avoid subjects having to walk too fast or jog. An exercise specialist will monitor you during all exercise-training sessions and supervise any necessary equipment adjustments. Each training session will begin with 5-10 minutes of light treadmill walking and will be followed by a 5-minute cool-down period on the treadmill. Each

treadmill walking session will last approximately 1 hour. The duration of the treadmill walking program will be 9 months.

Weight Lifting Programs: If you are assigned to either Group #2 or Group #3 you will be given a familiarization session with the MedX weight lifting machines. The weight lifting machines will be available in 2 locations: (1) at the Center For Exercise Science in the Florida Gym at the University of Florida or (2) at Medical Exercise Associates, Daytona Beach, Florida. You will do all of your weight training and testing at the facility where you enter the study (either Gainesville or Daytona Beach). Group #2 will be asked to perform weight lifting exercises 2 days per week on the following MedX machines: Leg Extension, Leg Curl, Lat Pull Down, Seated Row, Chest Press, Overhead Press, Biceps Curl, Triceps Pushdown, and Abdominal. These machines are designed to work all major muscle groups. Participants in Group #2 will also exercise on the clinical lumbar extension and leg press machines (total 11 machines). Group #3 will be asked to perform weight lifting exercises 2 days per week on the same MedX equipment as Group #2 but will not exercise on the clinical lumbar extension and leg press machines (total 9 machines). The initial resistance for each subject on each machine will be 50% of the participant's peak strength. You will be asked to complete one set of 8 to 12 repetitions per machine. Participants in Group #2 will also be asked to complete one set of 20 repetitions on the lumbar extension machine. The final repetition should require all of your strength to complete. When 12 repetitions are achieved (or 20 on the lumbar machine), the training weight will be increased 5% at the next training session. An exercise specialist will monitor each participant during all exercise-training sessions. Each training session will begin with 5-10 minutes of treadmill walking and will be followed by a 5-minute cool-down period on the treadmill. Each weight lifting session will last approximately 1 hour. The duration of the weight lifting programs will be 9 months.

Blood drawing: Each time you have a bone scan performed, we will draw 20 ml (2 tablespoons) of blood from a vein in your arm. We will use the blood samples to help determine if you are adding new bone, losing bone, or remaining the same. Additional blood samples will be drawn during the week prior to your 4½-month and 9-month bone scans. These blood samples will be drawn both before and after one regularly scheduled exercise session. A total of 40 ml (4 tablespoons) will be drawn on these days. These blood samples will be used to determine changes in circulating levels of hormones that influence muscle size and strength.

7. What are the possible discomforts and risks?

Strength Testing or Training. A potential source of discomfort to you will be from muscle soreness. This may occur 24-48 hours after a strength test or exercise session and usually the soreness goes away after a few days. Exercise on the MedX machines may cause you to experience some soreness in the muscles of the legs, arms and torso. Exercise on the MedX lumbar extension machine may cause you to experience some

soreness in the low back muscles. There is a possibility that a muscle strain could occur which could halt training. In rare instances, some subjects may experience temporary discomfort from lap and leg restraints on the MedX lumbar machine. The risks associated with exercising on the MedX machines will be minimized by: a) practice on the machines with light weight to familiarize subjects with the testing/training process; b) increasing the exercise weight by only 5% when subjects are able to complete more than 12 repetitions (or 20 repetitions on the lumbar machine); c) supervising all exercise sessions with trained personnel. Weight lifting exercise could aggravate or worsen an existing medical problem that is known or unknown to you. You are encouraged to discuss participating in this study with your personal physician or clinician.

Treadmill Walking. A possible source of discomfort to participants assigned to the treadmill walking group is muscle soreness. This may occur 24-48 hours an exercise session and usually the soreness goes away after a few days. The risk associated with treadmill walking will be minimized by having participants achieve an exercise heart rate no greater than 80% of peak heart rate and by having an exercise specialist supervise each of your treadmill sessions. Treadmill walking exercise could aggravate or worsen an existing medical problem that is known or unknown to you. You are encouraged to discuss participating in this study with your personal physician or clinician.

Bone Scan. There is a small X-ray dosage during the bone thickness measurements. The effective radiation dose equivalent (H_E) is about 2.5 μ Sv during a total body scan. This exposure is approximately 0.3% of the average annual per capita background radiation exposure in the U.S.

Blood Drawing. Blood samples will be drawn from a forearm vein before you begin your exercise program and after 4 ½ and 9 months of the exercise program. The risks of drawing blood from a vein include discomfort at the site of the injection; possible bruising and swelling around the injection site; rarely an infection; and, uncommonly, faintness from the procedure. This risk will be minimized by the use of sterile aseptic techniques. The principal investigator has 15 years of experience in drawing venous blood samples from human subjects.

Throughout the study, the researchers will notify you of new information that may become available and might affect your decision to remain in the study.

If you wish to discuss the information above or any discomforts you may experience, you may ask questions now or call the Principal Investigator or contact persons listed on the front page of this form.

8a. What are the possible benefits to you?

Potential benefits to you include a free evaluation of your bone density, which would otherwise be very costly. You will also gain the experience of participating in a

supervised exercise program. This experience may be useful to you in subsequent exercise programs. Also, you should experience an increase in muscle strength and/or bone thickness, which may reduce complications associated with postmenopausal osteoporosis.

8b. What are the possible benefits to others?

The knowledge gained from this study may be used to reduce osteoporosis and its complications in other postmenopausal women. We may gain insight into what types of exercise programs are most effective in increasing bone density. We may also learn methods to predict changes in bone metabolism by looking at elements present in the blood.

9. If you choose to take part in this research study, will it cost you anything?

All of the testing procedures and the exercise programs will be provided at no cost to you.

10. Will you receive compensation for taking part in this research study?

No financial compensation will be given for participation in this study.

11. What if you are injured because of the study?

If you experience an injury that is directly caused by this study, only professional ^ care that you receive at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will have to be paid by you or your insurance provider. No other compensation is offered.

12. What other options or treatments are available if you do not want to be in this study?

Participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not influence current or future health care you receive at this institution.

13a. Can you withdraw from this research study?

You are free to withdraw your consent and to stop participating in this research study at any time. If you do withdraw your consent, there will be no penalty, and you will not lose any benefits you are entitled to.

If you decide to withdraw your consent to participate in this research study for any reason, you should contact: Dr. Randy Braith at (352) 392-9575 extension 1340, or Dr. Michael Fulton at (386) 258-9502.

If you have any questions regarding your rights as a research subject, you may phone the Institutional Review Board (IRB) office at (352) 846-1494.

13b. If you withdraw, can information about you still be used and/or collected?

Yes, any information collected while in the study may be used, with your permission, in the final analysis of the data.

13c. Can the Principal Investigator withdraw you from this research study?

You may be withdrawn from the study without your consent for the following reasons: (1) to protect your health or safety; (2) a change in medications that would affect the outcome of the study; (3) inability to continue the assigned exercise program.

14. How will your privacy and the confidentiality of your protected health information be protected?

If you participate in this research, your protected health information will be collected, used, and disclosed under the terms specified in sections 15 – 24 below. If the results of this research are published or presented at scientific meetings, your identity will not be disclosed.

15. If you agree to participate in this research study, what protected health information about you may be collected, used and disclosed to others?

To determine your eligibility for the study and as part of your participation in the study, your protected health information that is obtained from you, from review of your past, current or future health records, from procedures such as physical examinations, x-rays, blood or urine tests or other procedures, from your response to any study treatments you receive, from your study visits and phone calls, and any other study related health information, may be collected, used and disclosed to others. More specifically, the following information may be collected, used, and disclosed to others:

- Complete past medical history to determine eligibility criteria listed in informed consent
- Records of physical exams
- 3-day Food Diary
- Results from bone mineral density tests
- Results from laboratory tests on blood samples to determine new bone growth

16. For what study-related purposes will your protected health information be collected, used and disclosed to others?

Your protected health information may be collected, used and disclosed to others to find out your eligibility for, to carry out, and to evaluate the results of the research study. More specifically, your protected health information may be collected, used and disclosed for the following study-related purpose(s):

In the United States, 50% of women suffer bone fractures after the onset of menopause. Of the more than 1.5 million fractures that occur each year in postmenopausal women, over 300,000 are fractures of the hip and 700,000 involve the spinal vertebra. Most hip and vertebral fractures are caused by low bone thickness. No effective drug therapy is widely available to increase bone thickness in postmenopausal women. The purpose of this study is to determine the effects of different exercise programs on bone thickness. We believe that specific exercise programs will slow the loss of bone and, perhaps, even result in increased bone formation. Sixty healthy postmenopausal women will be included in the study. You are being asked to participate because you have experienced natural (non-surgical) menopause for at least one year and have not received hormone replacement therapy for at least six months.

17. Who will be authorized to collect, use and disclose to others your protected health information?

Your protected health information may be collected, used, and disclosed to others by

- the study Principal Investigator (*Randy W. Braith, Ph.D.*) and his staff
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures

- the University of Florida Institutional Review Board

Randy Braith, Ph.D.
 Michael Fulton, MD
 Kathy Howe, MS
 Mark Mering, BS

18. Once collected or used, who may your protected health information be disclosed to?

Your protected health information may be given to:

- the study sponsor (*The University of Florida*)
- United States and foreign governmental agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, and the Office of Human Research Protections
- Government agencies who are responsible for overseeing public health concerns such as the Centers for Disease Control and Federal, State and local health departments
- Malcolm Randall VA Medical center (Gainesville)

19. If you agree to participate in this research, how long will your protected health information be collected, used and disclosed?

The Principal Investigators will use and disclose your protected health information until the end of the study.

20. Why are you being asked to authorize the collection, use and disclosure to others of your protected health information?

Under a new Federal Law, researchers cannot collect, use or disclose any of your protected health information for research unless you allow them to by signing this consent and authorization.

21. Are you required to sign this consent and authorization and allow the researchers to collect, use and disclose (give) to others of your protected health information?

No, and your refusal to sign will not affect your treatment, payment, enrollment, or eligibility for any benefits outside this research study. However, you cannot participate in this research unless you allow the collection, use and disclosure of your protected health information by signing this consent/authorization.

22. Can you review or copy your protected health information collected, used or disclosed under this authorization?

You have the right to review and copy your protected health information. However, you will not be allowed to do so until after the study is finished.

23. Is there a risk that your protected health information could be given to others beyond your authorization?

Yes. There is a risk that information received by authorized persons could be given to others beyond your authorization and not covered by the law.

24. Can you revoke (cancel) your authorization for collection, use and disclosure of your protected health information?

Yes. You can revoke your authorization at any time before, during or after your participation in the research. If you revoke, no new information will be collected about you. However, information that was already collected may be still be used and disclosed to others if the researchers have relied on it to complete and protect the validity of the research. You can revoke by giving a written request with your signature on it to the Principal Investigator.

25. How will the researcher(s) benefit from your being in this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in scientific journals.

26. Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternatives to being in the study; and how the participant's protected health information will be collected used and disclosed:

Signature of Person Obtaining Consent and Authorization

Date

You have been informed about this study's purpose, procedures, possible benefits, and risks; the alternatives to being in the study; and how your protected health information will be collected, used and disclosed. You have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and disclosure of your protected health information as described in sections 14-24 above. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing

Date

APPENDIX B
STUDY HEALTH QUESTIONNAIRE

Name: _____ Date of Birth: _____

Address: _____ Phone number: (w) _____

_____ (h) _____

Screening Blood Pressure Measurement: _____ / _____

Please answer the following questions:

I. GENERAL HEALTH

- | | | |
|--|---|---|
| 1. Have you ever been told by a physician that you have Osteoporosis/Osteopenia? | Y | N |
| 2. Have you ever been told by a physician that you have a heart condition? | Y | N |
| 3. Have you or anyone in your immediate family had a heart attack, stroke, or cardiovascular disease before age 50? If "yes," please explain.
_____ | Y | N |
| 3. Have you ever been told by a physician that you have high blood pressure? | Y | N |
| 4. Have you ever been told by a physician that you have high cholesterol? | Y | N |
| 5. Have you ever been told by a physician that you have thyroid problems? | Y | N |
| 6. Have you ever been told by a physician that you have kidney disease? | Y | N |
| 7. Do you feel angina-like symptoms (pain or pressure in your chest, neck, shoulders, or arms) during or after physical activity? | Y | N |
| 8. Do you ever lose your balance because of dizziness? | Y | N |
| 9. Do you ever lose consciousness? | Y | N |
| 10. Do you consider yourself to be generally healthy? | Y | N |

11. Please list all of the **prescription medication** you are currently taking

Medicine:	Amount taken per day	Medicine:	Amount taken per day
a. _____	_____	f. _____	_____
b. _____	_____	g. _____	_____
c. _____	_____	h. _____	_____
d. _____	_____	i. _____	_____
e. _____	_____	j. _____	_____

12. Please list all of the **over-the-counter medicines or supplements** (including vitamins that you take regularly)

Item:	Amount taken per day	Item:	Amount taken per day
a. _____	_____	g. _____	_____
b. _____	_____	h. _____	_____
c. _____	_____	i. _____	_____
d. _____	_____	j. _____	_____
e. _____	_____	k. _____	_____
f. _____	_____	l. _____	_____

II. REPRODUCTIVE STATUS

13. How long has it been since you reached menopause? _____

14. Do you still have your ovaries? _____ **Y N**

a. If not, how old were you when they were removed? _____.

15. Have you ever been on hormone replacement therapy? **Y N**

a. If so, are you still taking hormone replacement therapy? **Y N**

- a. If you have previously taken hormone replacement therapy, but have since stopped, when did you stop taking hormone replacement therapy?

16. Have you ever taken osteoporosis medications? Y N

Which ones and for how long? _____

III. OSTEOPOROSIS/FRACTURE/BONE HEALTH SECTION

17. Please provide a list of bone fractures you have had within the past five years

Bone:	cause: (fall, accident, etc)	Year
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

18. Did a doctor tell you that any of these fractures were due to osteoporosis/osteopenia? Y N

19. Is your diet low in dairy products? Y N

20. Do you take calcium supplements? Y N
If so, how much per day? _____

21. In a typical week, how many alcoholic drinks do you consume? _____

22. Do you drink coffee, tea, or cola products routinely? Y N

About how much coffee, tea, or cola do you drink on an average day?

IV. EXERCISE SECTION

23. How many times per week do you generally exercise? _____

a. What type of exercise do you generally do? _____

b. In a typical week, how much time do you spend exercising? _____

24. Do you have any joint problems that would prevent you from exercising? **Y** **N**

25. Do you have any lower back problems that would prevent you from exercising? **Y** **N**

26. Do you have any other medical condition that might prevent you from exercising? (Diabetes, recent surgery, etc.) **Y** **N**

27. Do you currently smoke? **Y** **N**

28. Are you a former smoker? **Y** **N**
If so, how long has it been since you quit smoking? _____

29. What is your current height and weight? Height _____ Weight _____

30. Are there any other health related issues we should know about? _____

YOUR SIGNATURE

DATE

Recommended for Study: _____

Not Recommended for Study: _____

MEDICAL DIRECTOR

Date: _____

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BIOGRAPHICAL SKETCH

Kathleen S. Howe was commissioned as an officer in the United States Air Force following her graduation from the University of Central Florida in 1978. She spent the next twenty years serving in a number of positions in the United States Air Force before retiring as a Lieutenant Colonel in 2000. Following her retirement, she returned to the academic world to pursue her interest in bone metabolism. She has spent the past four years concentrating on her studies in exercise physiology and examining the effects of exercise interventions on bone density in healthy and clinical populations. She plans to continue research related to bone metabolism in her doctoral studies and hopes to teach and conduct research once her studies are complete.