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Aerobic Exercise and Bone Turnover in Trained and Untrained Premenopausal Women

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Aerobic Exercise and Bone Turnover in Trained and Untrained Premenopausal Women

by

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ABBREVIATIONS

BMD	Bone Mineral Density
VO_2 Max	Maximum volume of oxygen uptake
ELISA	Enzyme-linked Immunosorbent Assay
RIA	Radioimmunoassay
Ca^{2+}	Calcium ion
iPTH	Intact Parathyroid Hormone
P1NP	Procollagen Type 1 N-terminal propeptide
CTx	C-terminal telopeptide cross-links of Type 1 collagen
WBC	White blood cells
RBC	Red blood cells
HGB	Hemoglobin
HCT	Hematocrit
25-OH	25-Hydroxy

ABSTRACT OF THE DISSERTATION

Aerobic Exercise and Bone Turnover in Trained and Untrained Premenopausal Women

by

Michelle Prowse

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Regular weight-bearing exercise is recommended for healthy young adults to optimize bone mineral density (BMD) (Physical Activity Guidelines Advisory Committee, 2008). BMD alone however, is not a significant predictor of osteoporosis or fracture risk. While bone turnover markers are used clinically to assess efficacy of pharmacological intervention in individuals diagnosed with osteoporosis, they may also have a role in evaluating acute responses to exercise. The purpose of this dissertation was to compare two groups of premenopausal women, those which are sedentary (low fitness) versus those that are well-trained (high fitness), by BMD and bone turnover markers at rest and the acute response of these bone turnover markers to a single bout of 60 min of aerobic jogging (or brisk walking).

The first study included 21 trained and 14 untrained premenopausal women, mean VO_2 Max 42.2±5.3mL/kg/min and 27.9±3.3mL/kg/min respectively, who performed 60 min of jogging or brisk walking at an aerobic intensity, 70-80% maximum heart rate. Fasting venous blood samples were drawn at pre-exercise, post-exercise and 30 min post-exercise. All markers of bone turnover and hormones were adjusted for plasma volume shifting. Mean serum C-telopeptide of type 1 collagen (sCTx) was higher in the trained

group, but not significantly different between groups. Procollagen type 1 N-terminal propeptide (P1NP) was significantly higher in the trained group compared to the untrained group at post-exercise (76.0±5.0 vs 47.2±5.3µg/L, p<.001) and 30 min post-exercise (57.4±3.7 vs 42.6±5.0µg/L, p=.01). In both groups, P1NP significantly increased from pre-exercise to post-exercise and at 30mins post-exercise P1NP returned to pre-exercise levels. In both groups, total serum calcium (Ca^{2+}) and mean intact parathyroid hormone (iPTH) increased from pre-exercise to post exercise and at 30mins post-exercise total Ca^{2+} and iPTH returned to pre-exercise levels. 25-OH Vitamin D was significantly higher in the trained group compared to the untrained group at pre-exercise, post-exercise and 30mins post-exercise. In both groups, 25-OH Vitamin D significantly increased from pre-exercise to post-exercise.

The second study included 23 trained and 19 untrained premenopausal women, mean VO_2 Max 42.4±5.2mL/kg/min and 28.2±3.3mL/kg/min respectively. Age-matched fitness was determined by reference tables published by the American College of Sports Medicine. BMD was measured by DXA scan for arms, legs, pelvis, spine and total body. Fasting venous blood samples were drawn to measure sCTx, P1NP, total Ca^{2+}, iPTH, 25-OH Vitamin D and Cortisol. BMD was analyzed by independent t-test, adjusted for differences in body fat percentage and BMI. Spearman's correlations were calculated for age-matched fitness and hormones and markers of bone turnover. There were no significant differences in BMD at any of the body regions (p>.05). Age-matched fitness significantly correlated with P1NP (.393, p=.01), iPTH (-.316, p=.04), 25-OH Vitamin D (.429, p=.01), and Ca^{2+} (.447, p=.003).

Trained premenopausal women have a different bone turnover marker profile and greater response to exercise than untrained premenopausal women. Despite finding no significant differences in BMD between the groups, bone turnover markers of type 1 collagen were elevated in the trained group at rest and greater increases were observed post-exercise. Furthermore, age-matched fitness was significantly correlated with P1NP, iPTH, total Ca^{2+}, and 25-OH Vitamin D. This highlights the inherent value of weight-bearing aerobic exercise in promoting bone remodeling and osteogenic responses to the skeleton in premenopausal women, but with greater responses in individuals with higher fitness levels.

CHAPTER ONE

INTRODUCTION

The Bone Organ System

Bone Structure, Bone Cells and Bone Remodeling

Bone is a specialized connective tissue comprised of cortical (compact) and porous trabecular (spongy) bone. On the surface of the bone is the periosteum which comprises of an outer fibrous layer and the inner cambium layer consisting of progenitors of bone formation cells, the osteoblasts (Figure 1a).

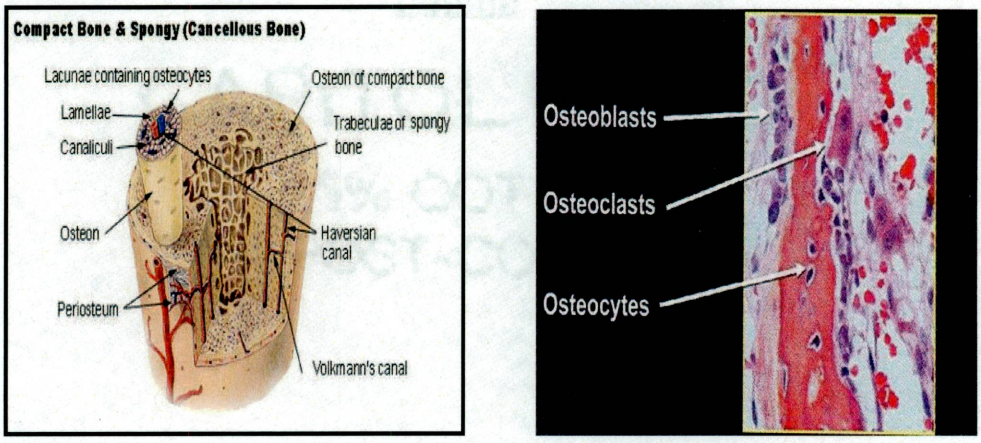

Figure 1. a) Bone structure and b) Bone cells (Adopted from Rubin and Strayer, 2008).

Ninety percent of bone is extra-cellular, known as osteoid, which includes an organic matrix of type 1 collagen, glucosaminoglycans and proteoglycans and an

inorganic matrix comprising of calcium hydroxyapatite-like crystals. These minerals increase the bone's stiffness, whereas the organic matrix provides flexibility (Chavassieux et al, 2007).

The cellular components comprise of osteoblasts, osteoclasts and osteocytes (Figure 1b). Osteoblasts are polygonal in shape and make up less than 5% of the cells on the bone surface. They are situated within trabecular bone and differentiate from stromal cell precursors in bone marrow. They are involved in the formation of bone by manufacturing the extracellular matrix (predominantly type 1 collagen) which subsequently mineralizes. Osteoblasts respond to a complex array of genetic and environmental factors. Activation of specific genes for skeletal development, predominantly *cba1* as well as several growth factors are known to activate osteoblasts. Activated osteoblasts then produce several cytokines such as RANK-Ligand, macrophage colony stimulating factor (m-CSF) and interleukin-6 and interleukin-11 which affect osteoclast recruitment (Figure 2).

Osteoclasts are multinucleated cells involved in bone resorption and calcium homeostasis. They are differentiated from hematopoetic stem cell precursors under the direction of factors that include cytokines, m-CSF and interleukins. The RANK/RANK-Ligand system is one of the major regulatory systems for osteoclast recruitment and action (Figure 2). The binding of RANK from the osteoclast precursor to its ligand on the osteoblast RANK-Ligand activates the osteoclast to adhere to bone. To remove bone, osteoclasts become polarized, form a ruffled membrane, adhere tightly to the bone matrix via α_V-β_3-integrin mediated binding and then secrete acid (H^+ ATPase) to dissolve hydroxyapatite and proteases Capthesin K for matrix protein digestion.

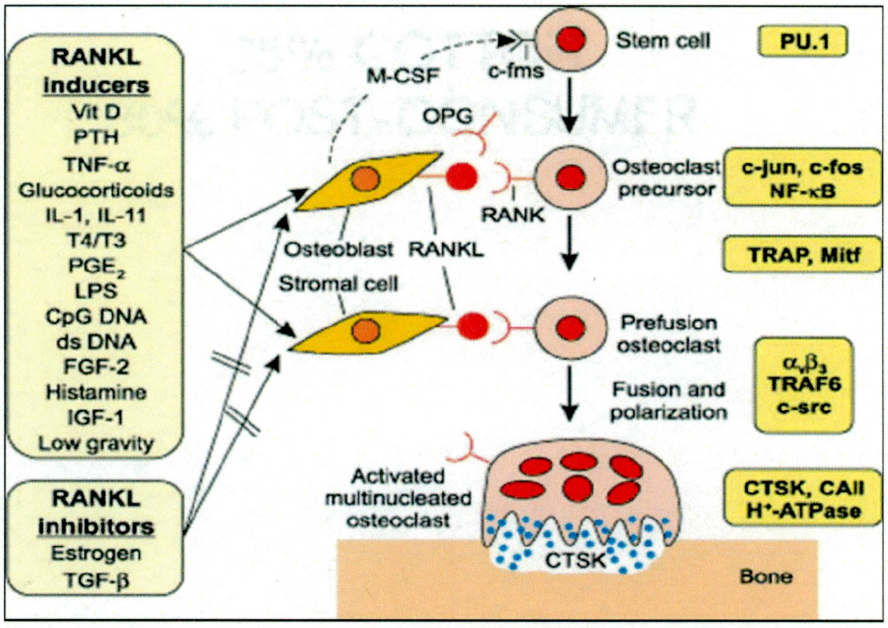

Figure 2. The OPG/RANK/RANK Ligand Pathway (Adopted from Kierszenbaum, 2007).

Osteocytes are ellipsoid, have multiple cytoplasmic extensions called dendrites and include 90-95% of all bone cells in the adult skeleton. Osteocytes are regularly dispersed throughout the mineralized matrix, especially in cortical bone. These cells are connected to each other and other cells on the bone surface, however, their dendrites are in contact with the bone marrow (Kamioka, 2001, cited Bonewald and Johnson, 2008) which enable them to recruit osteoclast precursors to stimulate bone resorption (Baylink et al, 1973) and to regulate mesenchymal stem cell differentiation (Heino et al., 2004, cited Bonewald and Johnson, 2008). They are thought to respond to mechanical strain by sending signals of resorption or formation (Lanyon, 1993).

Type 1 collagen fibers consist of three polypeptide chains which form a triple helix cross-linked by hydrogen bonding between hydroxyl groups on hydroproline and other charged residues such as intrachain water and aldehyde derived groups. Cross-links

are located between the telopeptide and helical region (Figure 3). The orientation of collagen fibers is important in determining the mechanical properties of bone and is related to the direction of load.

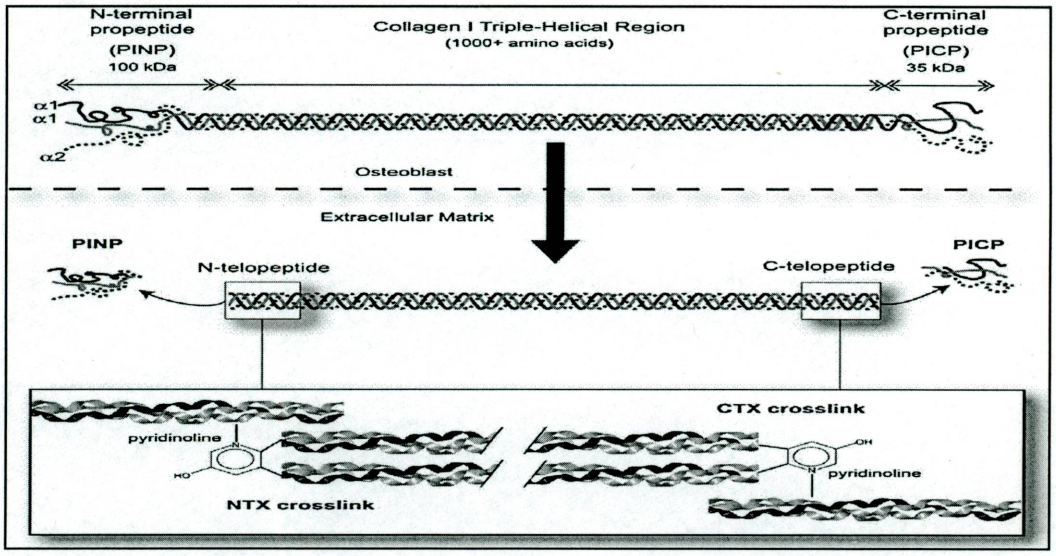

Figure 3. Molecular basis of type 1 procollagen and collagen formation and degradation (Adopted from Hermann and Siebel, 2008).

Type 1 collagen is derived from type 1 procollagen, which is secreted by osteoblasts. Procollagen polypeptide is produced in the endoplasmic reticulum of the osteoblast by hydroxylation, glycosylation and disulfide bond formation. Interchain disulfide bonds between the C-terminal propeptides of the three procollagens align the chains, initiate the formation of the triple helix and this process continues toward the N-terminus. These N- and C-terminal extensions are removed by specific proteases during the conversion of procollagen to collagen (Goldberg, 1975). The N-terminal extension of procollagen (P1NP) and a degradation product of the C-telopeptide of α1 chain of type 1

collagen (CTx) mature type 1 collagen have been explored as biomarkers in the investigation of growth and bone metabolism (Eastell and Hannon, 2008), as well as the evaluation and management of metabolic diseases (Miller et al, 1999).

Bone Remodeling and Mechanotransduction

Bone remodeling is the continuous turnover of bone matrix and minerals by bone resorption and formation in the skeleton. In adulthood, the purpose of bone remodeling is to maintain bone strength by removal of microdamage (Chavassieux et al, 2007). In both cortical and trabecular bone, the remodeling process is initiated by the activation of osteoclasts, followed by the filling in of the resorptive cavity by bone deposited by osteoblasts. Under normal conditions, the remodeling process of resorption is closely followed by formation and results in no change of bone mass.

The process of bone remodeling is adaptive, meaning that bone is remodeled to resist and withstand average daily loads. The osteoblasts and osteocytes sense biochemical signals from mechanical loading and these transduction pathways are still not clearly understood. Mechanical loading is believed to promote osteoblast differentiation and proliferation, collagen secretion and mineralization through a process called mechanotransduction. Scott et al, (2008) describe that mechanotransduction may be a result of four overlapping mechanisms which include 1) Stretch-activated Ca^{2+} channels, IP3 and cAMP dependent pathways, 2) Integrins and CD44 receptors, 3) G-proteins in the lipid bilayer and 4) Deformation of the cytoskeleton. In addition, these signaling pathways appear to coincide with other growth factors and hormones of systemic regulation.

For mechanical forces to have an osteogenic effect, the stress to bone must be unique, variable and dynamic in nature. Static loading of bone (i.e. single sustained force application) does not trigger the adaptive response that occurs with dynamic loading (Burr et al, 2002). Bonewald and Johnson, (2008) report that tissue deformation with dynamic loading can lead to perturbation of bone fluid and cell membrane deformation, and that this fluid flow generates a shear stress contributory to Mechanotransduction.

Hormonal Regulation of Bone Remodeling

The physiological balance of calcium metabolism and of bone remodeling is under regulation of systemic hormones, especially calcium-regulating hormones, parathyroid hormone (PTH), $1,25(OH)_2$ Vitamin D and calcitonin.

The primary role of PTH is the maintenance of adequate levels of plasma ionized calcium. Serum Ca^{2+} levels normally average 9.5mg/dL (Kierszenbaum, 2007). Secreted by the parathyroid glands in response to low plasma ionized calcium, PTH promotes bone resorption by releasing calcium from bone, inducing renal conservation of calcium and excretion of phosphate, and by indirectly enhancing intestinal calcium absorption by increasing the renal production of the active Vitamin D metabolite $1,25(OH)_2$ Vitamin D. PTH binds to the cell surface of the osteoblast to regulate the synthesis of three proteins essential for the differentiation and function of osteoclasts: m-CSF, RANK-Ligand, and Osteoprotegerin. There is some evidence of increased fracture risk in women with studies supporting cortical thinning with increased basal PTH (Dempster et al, 1995) and that chronic hypersecretion of PTH induces bone loss (Ljunghall et al, 1994 cited Brahm et al, 1997). However, PTH may not always have catabolic effects on the skeleton. There

are studies in post-menopausal women that demonstrate the anabolic effects of low doses of PTH injections result in increased bone size and preservation of skeletal microarchitecture (Ma et al, 2006, cited Raisz, 2007).

Vitamin D_2 (ergocalciferol) is formed in the skin by the conversion of 7-dehydrocholesterol to Vitamin D_3 (cholecalciferol) following exposure to ultraviolet light. Cholecalciferol is then absorbed into the blood circulation and transported to the liver where it is converted to 25-OH Vitamin D by the addition of a hydroxyl group to the side chain. Low Ca^{2+} levels stimulate the enzymatic activity of mitochondrial 1α-hydroxylase to add another hydroxyl group to 25-OH Vitamin D to form $1,25(OH)_2$ Vitamin D, the active form of Vitamin D. The main function of Vitamin D is to stimulate calcium absorption by the intestinal mucosa by synthesizing Ca^{2+} binding protein. The interrelationship of Vitamin D and PTH in calcium metabolism is demonstrated in a study by Grey et al, (2005). They found that mild hyperparathyroidism patients with Vitamin D deficiency (average 20ng/mL) supplemented with Vitamin D_3 50,000 IU per week for the first month, and then 50,000 IU monthly for 12 months resulted in a 25% decrease in serum PTH levels. A meta-analysis by Bischoff-Ferrari et al, (2005) showed that supplementation of Vitamin D between 700-800 IU/d appears to reduce the risk of hip and non-vertebral fractures in ambulatory or institutionalized elderly persons.

Estradiol is the major steroid produced by follicular cells under stimulation of follicle stimulating hormone (FSH). The effects of estrogen in bone are of interest in relation to loss of bone after the menopause in women and the therapeutic use of estrogen to prevent this. Estrogen has been shown to stimulate Calcitonin production, resulting in a decreased bone resorption as well as enhanced availability of 1,25 Vitamin D_3 and

subsequent increased calcium absorption. Estrogen receptors exist in bone, although in low concentrations. Estrogens can affect the proliferation of osteoprogenitor cells and osteoblast-like cells. Although it is clear that estrogen deficiency results in accelerated bone resorption at any age, the precise mechanisms have still not been established (Raisz, 2007). Recent studies indicate that estrogen may act at multiple sites – on both hematopoietic precursors of the osteoclasts and the osteoblast-osteoclast interaction that regulates bone resorption. Thus, estrogen administration can decrease bone resorption by decreasing the ability of marrow cells to respond to stimulation by receptor activator RANK-Ligand and by decreasing the expression of RANK-Ligand in marrow cells (Eghbali-Fatourechi et al, 2003; Taxel et al, 2008).

Endogenous corticosteroids may also play a role in bone physiology. Human osteoblastic cells *in vitro* express an enzyme (11β-hydroxysteroid dehydrogenase type 1) which converts hormonally inactive cortisone to active Cortisol (Thomlinson et al, 2004) and attenuates proliferation and differentiation associated with age-related bone loss (Cooper et al, 2002). Cooper et al, (2005) investigated 135 postmenopausal women and found a negative correlation between serum cortisone and spine BMD ($r=-0.18$, $p<.05$). In healthy elderly men, integrated 24-h Cortisol levels negatively correlated with lumbar spine BMD ($r=-0.37$, $p<.05$) and femoral neck BMD ($r=-0.31$, $p=.06$) (Dennison et al, 1999). Another study did not find any association between serum Cortisol and BMD in postmenopausal women (Acar et al, 1998). To our knowledge, the relationship of Cortisol and markers of bone turnover in premenopausal women has not been investigated.

Osteoporosis

Bone Changes associated with Osteoporosis

Osteoporosis is a skeletal disease characterized by low bone mass and structural deterioration of bone leading to bone fragility and increased susceptibility to fractures (Kanis, 1994). Bone fragility is the result of changes in the material and structural properties of bone rather than simply reduced amount of bone (Chavassieux et al, 2007). Its pathological basis includes a combination of genetic predisposition, subtle alterations in systemic and local hormones, as well as environmental influences (Eastell and Hannon, 2008). Age-related changes in collagen content and structure also play an important part in skeletal fragility with additional glycosylation and cross-linking resulting in collagen losing its elasticity. In postmenopausal women, a high remodeling rate contributes to bone fragility by reducing the time available for secondary mineralization. Bone is removed and replaced with new, less densely mineralized bone, which reduces its material stiffness. Collagen composition is also altered by impairing isomerization, maturation, and cross-linking. A negative bone balance caused by an increase in volume of bone resorbed, a decrease in the volume of bone formed or both, accelerates bone loss and structural decay producing trabecular thinning, tunneling in the trabeculae, cortical thinning, and porosity (Chavassieux et al, 2007) (Figure 4).

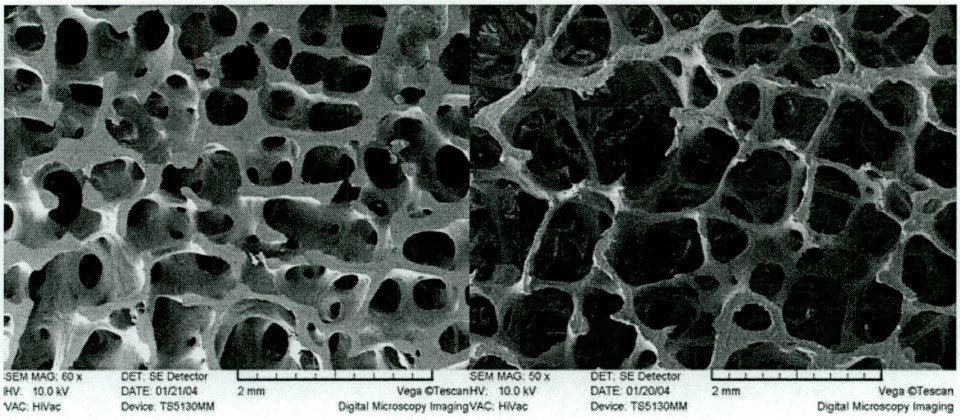

Figure 4. Trabecular thinning and tunneling associated with osteoporosis. On the left is a healthy 22 year old woman; on the right is an 80 year old woman with osteoporosis. (Adopted from International Osteoporosis Foundation, 2010).

Assessment

Conventionally, osteoporosis is diagnosed by the presence of fragility fracture or by dual energy X-ray absorptiometry (DXA) according to T-score, which represents the standard deviation (SD) difference between the BMD of that individual and that of a young-adult reference population (Lewiecki and Watts, 2008). Osteoporosis is clinically determined by a T-score less than -2.5 whereas osteopenia is a T-score between than -1.0 and -2.5. In premenopausal women, z-scores are used when referencing individuals to their own young-adult population. While DXA is typically the "gold standard" in clinical diagnosis of osteoporosis and osteopenia, measurement of BMD is limited in that it cannot determine the structure, geometry and mechanical properties of bone, all of which are known to affect bone strength and to change with age (Forwood, 2001).

Other factors, such as markers of bone remodeling, are major determinants of bone strength (Chavassieux et al, 2007). There are currently more than ten different bone

turnover markers that are commercially available. The most sensitive and specific markers for the formation and degradation of type 1 collagen are P1NP and CTx (Eastell and Hannon, 2008). Assay features of biochemical markers have significantly improved over the past few years, however, the clinical performance of these markers differs significantly depending on the clinical situation. Hermann and Seibel, (2008) provide a comprehensive review of two of the collagen degradation markers, CTx and NTx, which describe several strategies for reducing variability in the performance of these assays. These include pre-sampling fasting, correct handling, processing and storage of specimens and consideration diurnal variation (highest values of CTx in the morning, lowest values in the afternoon).

The National Osteoporosis Foundation (NOF) has suggested that the strongest predictions of fracture risk may result from a combination of BMD measurement and bone turnover markers, since high bone turnover has been associated with low BMD in post-menopausal women (Raisz et al, 2009) and high bone resorption markers relative to bone formation markers have a detrimental effect on bone microarchitecture and fragility (Seeman, 2005, cited Eastell and Hannon, 2008). There is less evidence for the biomarkers of bone turnover in premenopausal women. Cross-sectional studies have shown bone turnover is higher in early adulthood compared to later premenopausal years, and decreases with age (Adami et al, 2008; Glover et al, 2008) but rapidly increases after menopause (Garnero et al, 1996) with a substantial increase in CTx (Milliken et al, 2003).

Prevention and Treatment

Typically low BMD is not identified until women are well beyond menopause as DXA scans are not routinely prescribed for women under the age of 50 years. Treatment after diagnosis commonly involves the prescription of drugs that inhibit bone resorption which include bisphosphonates, estrogens and calcitonin (Eastell and Hannon, 2008).

Osteoporosis, the most common bone disease, afflicts 10 million Americans over the age of 50 years (Raisz et al, 2009). The National Institutes of Health's Consensus Conference on the prevention, diagnosis, and therapy of osteoporosis recommended that reducing the prevalence of osteoporosis and osteopenia in older women may be realized by maximizing BMD during the premenopausal years. As a result of maximizing BMD during this time period, a greater absolute amount of BMD may be maintained during the postmenopausal years despite the inevitable loss of some BMD during this period (Kelley and Kelley, 2004).

Physical activity is the only intervention that can potentially both increase bone mass and strength and reduce the risk of falling in older populations (Eastell and Hannon, 2008). Yet, because peak bone mass is thought to be attained by the end of the third decade, the early adult years may be the final opportunity for its augmentation (Raisz et al, 2009).

The guidelines for promoting bone health were published in 2004 after a large meta-analysis on BMD in healthy adults (Raisz et al, 2009). Evidence from small randomized controlled trials, and large observational studies, recommend the following exercise prescription for healthy adults:

1. **Mode:** weight-bearing endurance activities (tennis, stair climbing; jogging, at least intermittently during walking, activities involving jumping and resistance exercise
2. **Intensity:** moderate to high, in terms of bone loading forces
3. **Frequency:** weight-bearing endurance activities 3-5 times per week; resistance exercise 2-3 times per week
4. **Duration:** 30-60mins/day of combined weight-bearing endurance activities, activities that involve jumping and resistance exercise that targets all major muscle groups

Proper nutrition is an important consideration to bone health. Adequate calcium and vitamin D intake are critical to maintaining health and preventing illnesses, including osteoporosis. It is well understood that calcium plays a key role in building stronger denser bones early in life and keeping them strong throughout the lifespan. As described previously, adequate Vitamin D is necessary for the absorption of calcium in bone. Yet, findings suggest that 70 percent of Americans over age 2 years do not get enough calcium on a daily basis (recommended 1000mg for adults under age 50 years) (Briefel and Johnson, 2004). Similarly, current research suggests that a minimum of 25 percent of adolescents and adults in the United States may not have sufficient Vitamin D (Richardson, 2005). Currently, the United States government is undertaking a new review of evidence, which precedes any change in recommended intakes of Vitamin D, currently 400IU to 600IU (Raisz et al, 2009). In addition, protein intake may be

important for achieving optimal bone mass and strength (Mundy, 2006). Because of extensive post-translational modification of amino-acids in the collagen molecule (e.g. Cross-linking, hydroxylation), many of the amino acids released in bone resorption cannot be recycled. Hence, bone turnover needs a continuing supply of fresh dietary protein. Moreover, studies have shown protein increases IGF-1, which is trophic for bone (Bonjour et al, 1997 cited Marcus et al, 2008). Yet, the evidence that protein intake effects bone health is limited. Two currently accepted but contradictory theories exist relating to protein intake: 1) Increases bone resorption as protein intake increases urinary calcium loss and 2) Promotes bone preservation as protein aids recovery from hip fracture and slows age-related bone loss (Marcus et al, 2008).

CHAPTER TWO

LITERATURE REVIEW

This section will include the literature on bone turnover markers in premenopausal women and the potential modulation of bone metabolism with aerobic exercise in this population. The first section reviews studies with cross-sectional designs in premenopausal women which have attempted to establish a norm reference range for bone turnover markers in this population. The second section reviews studies which have investigated the effects of bone turnover markers with weight bearing aerobic exercise in premenopausal women. While some of the studies have included additional biomarkers of bone turnover, the evidence will be reported only for selected biomarkers (P1NP (or P1CP), CTx, PTH, Ca^{2+}, 25-OH Vitamin D and Cortisol) to enable comparison with subsequent sections of this dissertation.

Cross-sectional Studies

DeSouza et al, (2008) divided 44 trained premenopausal women into four groups (mean VO_2 max ranging from 41.9±3.1 to 47.7±0.8) according to estrogen deficiency (<35ng/mL) or non-deficiency (determined by menstrual status and estrone glucuronides), and energy deficiency or non-deficiency (based on resting energy expenditure). They found that urinary CTx (uCTx) was significantly higher in the estrogen deficient + energy deficient group compared to the other groups. P1NP was

significantly lower in the estrogen deficient + energy deficient group compared to the other groups. This study provides some evidence to support the negative impact of insufficient estrogen and inadequate dietary intake in trained premenopausal women by elevation of bone resorption markers and suppression of bone formation markers. However, due to the small sample size (7-8 per group), more studies are needed to validate these findings.

Adami et al, (2008) compared 530 premenopausal women according to how much time they reported exercising each day. The three groups included sedentary (no exercise), less than 30mins/day or greater than 30mins/day. One blood draw was obtained to measure sCTx and P1NP. P1NP levels were significantly elevated in the group reporting the most physical activity and lowest in the group reporting no physical activity. There were no significant differences in sCTx between the groups. This study provides some insight into differences in bone formation markers between groups of varying levels of exercise participation. In this study, the type of exercise was not reported and level of physical activity was dependent on a self-reported measure.

Another study by Adami et al, (2008) investigated 641 premenopausal women in order to define normal reference values for bone turnover markers. The subjects were divided into 5 year age categories, between 20 and 50 years of age. Both P1NP and sCTx decreased with age, with the highest concentration of bone turnover markers in the 20-25 year old age group (P1NP 53.2±17.7 and CTx 490±200) and the lowest concentration in the 45-50year old group P1NP 35.3±13.4 and CTx 260±130). Both BMI and age were negatively correlated with the bone turnover markers.

De Papp et al, (2007) investigated P1NP, sCTx, sPTH, Ca2+, and 25-OH Vitamin D levels and BMD in 237 premenopausal women. Mean values for the bone turnover markers were P1NP 39.5, sCTx 280, sPTH 48.3, Ca2+ 9.3, and 25-OH Vitamin D 37.0. The P1NP and sCTx values support the findings of Adami et al, (2008) as well as additional insight into PTH, Ca2+ and 25-OH Vitamin D values. BMD measurements were subdivided by femoral neck T-scores and there were no significant differences in the bone turnover markers between groups. A questionnaire was given to assess the use of calcium supplements and frequency of exercise per week but there was no further analysis reported of these factors with respect to the bone turnover markers.

Glover et al, (2008) investigated P1NP, sCTx, PTH, and 25-OH Vitamin D levels in 153 premenopausal women to determine normal reference values and also identify determinants of these bone turnover markers. Mean values for the bone turnover markers were P1NP 35.9±16.4, sCTx 300±150, PTH 75.8±31.5, and 25-OH Vitamin D 39.8±37.8. These values were similar to that of Adami et al, (2008) and De Papp et al, (2007). Furthermore, mean bone turnover markers were significantly higher in younger premenopausal women 30-35 years compared to those 35-45 years of age. This study found no significant differences in P1NP or sCTx in those that were Vitamin D deficient, insufficient or replete or between those that reported exercising regularly and those that did not.

In summary, these cross-sectional studies provide reference values in large groups of premenopausal women and the mean values of P1NP, sCTx and 25-OH Vitamin D were similar between studies. The sCTx findings of De Souza et al, (2008) in trained premenopausal women highlight the need for accurate assessment of dietary intake and

estrogen status in addition to biomarkers of bone formation. There appears to be a wider variability in PTH between studies. This could be due to poor methods of dietary assessment, diurnal variation (Nielsen et al, 1991) and different levels of physical activity between subjects. Currently, there are no cross-sectional studies in premenopausal women which have compared different defined levels of fitness to basal levels of markers of bone turnover. Some of the above studies have measured the frequency of physical activity, yet they are limited as they are based on non-standardized, brief self-reported questionnaires.

Exercise Intervention Studies

Evans et al, (2008) investigated the effects of a 4-month military program including running, marching, jumping, battle drills, walking and standing for prolonged periods of time on 153 premenopausal women (mean age 19±1.0 years). Fitness was determined by a treadmill test before and after the 4-month intervention with the mean VO_2 Max 36.7±6.2 and 39.7±5.8 respectively. Blood draws were taken at baseline, 2-months, and 4-months and P1NP, sCTx, PTH, Ca2+, and 25-OH Vitamin D levels were measured. P1NP levels increased 20.6% from 0 to 2 months and dropped from 2 to 4 months. Similarly, CTx increased from 0 to 2 months and decreased from 2 to 4 months. PTH decreased from 0 to 2 months and returned to baseline during the 2 to 4 month period. The change in PTH was positively associated with CTx from 0 to 2 months. Calcium decreased from 0 to 2 months and returned to baseline during the 2 to 4 month period. The change in serum calcium was negatively associated with P1NP and CTx from 0 to 2 months and 2 to 4 months. 25-OH vitamin D decreased from 0 to 2 months

then increased from 2 to 4 months. These findings provide some evidence for the ability of P1NP and CTx to increase with exercise training, and these changes occurred in the first 2 months of training. The limitations of this study include the poor reproducibility of the exercise intervention in non-military populations, lack of dietary assessment and lack of assessment regarding the stage of the menstrual cycle during the three time points measured.

Toson et al, (2006) studied 9 sedentary (untrained) premenopausal women (mean age 28±2.2 years) comparing the effect of brisk walking with or without weight-lifting on bone turnover markers. The exercise was conducted on two separate days including 30mins of walking on a treadmill for 60-85% predicted maximum heart rate with or without a 5kg back pack. On a third day, subjects did not exercise during the same interval of time and served as a control group. Blood samples were collected at baseline, 30mins (post-exercise), 45mins and 24hrs post-exercise. P1NP and PTH were measured. No statistical differences were observed for P1NP. For the walking group without the 5kg back pack mean PTH significantly increased from baseline to post-exercise, but returned to baseline at 45mins. Mean PTH for subjects walking with the 5kg backpack also increased from baseline to post-exercise but the differences were not significant. These findings suggest that 30mins of brisk walking can acutely increase PTH but not P1NP in untrained premenopausal women. This study however, is limited by a very small sample size and there is no mention of correction for plasma volume shifting.

Rudberg et al, (2000) investigated 7 premenopausal women (mean age 23±2 years) who all took low-dose contraceptives and reported some recreational exercise, but not at an athletic level. The exercise intervention involved outdoor jogging for 30-

40mins at a perceived even pace for each individual. Non-fasting blood samples were obtained at baseline, post-exercise and 20mins post-exercise. PTH and Ca2+ were measured. PTH significantly increased from baseline to post-exercise then returned to baseline at 20mins post exercise. Ca2+ did not change across time. This study supported the findings of Tosun et al, (2006) in that PTH acutely rises with moderate exercise in premenopausal women and this study did correct for changes in plasma volume. Care should be taken with interpretation of these findings due to the low sample size and lack of fasting blood samples. In addition, this study was limited by the poor standardization of the "self-paced jogging" intensity and varied time of exercise between subjects.

Brahm et al, (1997) investigated 10 premenopausal women (mean age 30 years) and 10 men (mean age 28 years) of variable fitness (mean VO_2 Max 46.8 for women and mean VO_2 Max 56.2 for men). Exercise included approximately 35mins on a treadmill, three 10min progressive submaximal loads corresponding to 47-76% VO_2 Max followed by 4-5mins of maximal effort until exhaustion. Non-fasting blood samples were obtained at baseline, post-exercise, 30mins post-exercise and 24hrs post-exercise. P1CP, PTH, and Ca2+ were measured as well as total body BMD, and regional BMD. Mean P1CP significantly increased post-exercise and returned to baseline at 30mins post-exercise. Mean PTH increased from baseline at post-exercise and 30mins post-exercise. Mean Ca2+ slightly increased from baseline to post-exercise but returned to baseline at 30mins post-exercise. VO_2 Max was inversely related to basal concentration of PTH and remained significant after adjustment for age and sex (β=-0.9, p=.03). Arms and femoral neck BMD was inversely related to basal Ca2+ levels. This study is the only study to find a relationship between bone mass and VO_2 Max in premenopausal women. Similar

to the other studies, the findings demonstrate the ability for significant increases in bone turnover markers with exercise. Limitations of this study were the small sample size, and the inclusion of male subjects in that the analyses should have been done gender specific.

Thorsen et al, (1997) investigated 14 untrained premenopausal women (mean age 25.2±0.6 years) who underwent 45mins of outdoor jogging at 50% of their predicted HR Max. Non-fasting blood samples were collected at 15mins prior to exercise, 1 hr post-exercise, 24hrs post-exercise and 72hrs post-exercise. P1CP, PTH and Ca2+ were measured. A significant reduction in mean P1CP at 1hour was followed by an increase at 24hrs and 72hrs. Mean PTH increased 24hrs and 72hrs post-exercise compared with baseline. Mean Ca2+ decreased at 1hr post-exercise and 72hrs post-exercise compared with baseline. Estradiol and Cortisol were also tested in this study. Estradiol levels for all subjects were within normal values. Cortisol decreased significantly 1hr post-exercise but was unchanged at 24 and 72hrs post-exercise. This study's findings were concurrent with the previous studies for PTH and Ca2+ when comparing baseline and post-exercise. However, contrary to the findings of the other studies, the bone formation marker P1CP decreased post-exercise.

In summary, with the exception of Evans et al, (2008), the studies on premenopausal women investigating the effects of bone turnover markers with exercise had low sample sizes. There were also many blood sampling methodology errors which could have impacted the reported concentrations of the bone turnover markers. Brahm et al, (1997), Thorsen et al, (1997) and Rudberg et al, (2000) did not include fasting in their blood sampling which could have increased the variability of the bone turnover markers. Tosun et al, (2006) did not adjust for plasma volume shifting which also could lead to a

misrepresentation of the true concentrations of the bone turnover markers. Finally, the findings of the studies mentioned above enable only weak comparisons due to methodological differences in the exercise intervention (intensity, duration and mode). There is either limited or no reporting of factors known to affect bone metabolism such as level of physical activity participation, dietary intake and menstrual status. More vigorous studies are needed to determine the levels of bone turnover markers in premenopausal women with different and defined fitness levels, and the acute effects of aerobic exercise according to the current recommended exercises guidelines for promoting bone health.

CHAPTER THREE

PUBLISHABLE PAPER I

THE EFFECT OF A 1 HOUR AEROBIC RUN AT 70-80% MAX HR ON CTX AND P1NP BIOMARKERS OF BONE TURNOVER IN PHYSICALLY TRAINED AND UNTRAINED PREMENOPAUSAL WOMEN

Abstract

Introduction: Few studies have examined the effect on biomarkers of bone turnover in exercising premenopausal women. The purpose of this study was to compare low versus high fitness premenopausal women biomarkers of bone resorption C-Telopeptide of type 1 collagen (sCTx) and bone formation Procollagen N-terminal propeptide (P1NP) at rest and response to aerobic exercise. Methods: 21 trained and 14 untrained women, mean VO_2 Max 42.2±5.3 and 27.9±3.3mL/kg/min, performed a 1 hr exercise at 70-80% max HR. Blood concentrations were determined at pre-exercise, post-exercise and 30 min post-exercise for P1NP, sCTx, Ca2+, iPTH, 17β-Estradiol and 25-OH Vitamin D and adjusted for plasma volume shifts. Results: Higher CTx levels were observed in the trained group at all time points, but not significantly different between groups ($p \geq .05$). P1NP was significantly higher in the trained group versus the untrained at post-exercise (76.0±5.0 vs 47.2±5.3μg/L, $p < .001$) and 30 min post-exercise (57.4±3.7 vs 42.6±5.0μg/L, $p = .01$). In both groups, P1NP significantly increased from pre-exercise to post-exercise. In both groups, Ca2+ and iPTH increased from pre-exercise

to post exercise and by 30mins post-exercise returned to pre-exercise levels. 25-OH Vitamin D was significantly higher only in the trained group at all time points. Conclusions: We observed greater sCTx and PINP blood levels in the premenopausal physically trained group at rest and at post-exercise compared to the untrained group. This supports the significant role that appropriate weight-bearing aerobic exercise has in the promotion and benefit of bone remodeling in premenopausal women.
Keywords: Bone turnover biomarkers, CTx, exercise, fitness, P1NP, premenopausal

Introduction

Osteoporosis is a skeletal disease characterized by low bone mass and structural deterioration of bone leading to bone fragility and increased susceptibility to fractures (Kanis, 1994). The most recent data estimate that 2 million fragility fractures occurred in the United States (US) in 2005, and if this trend continues there will be over 3 million by 2025 (Johnell and Kanis, 2006). Therefore, there is a potential need for preventative interventions in premenopausal women.

In healthy adults, US recommendations for optimal bone health include weight-bearing exercise for 30-60 min at a moderate to vigorous intensity, at least 3-5 times per week (Physical Activity Guidelines Advisory Committee, 2008). Exercise studies to date have shown approximately 1-2% increases in BMD per year which is the equivalent in preventing up to 1-4 years of bone loss post-menopause (Shea et al, 2004). This relatively small change in BMD, over time, has led to the development of biomarkers which are able to determine alterations in bone matrix turnover (i.e. ratio of collagen synthesis and bone formation vs. collagen degradation and bone resorption). The collagen fibers in bone provide the ductability and ability to absorb energy (Viguet-Carrin et al, 2006). Alterations of collagen properties can therefore affect the mechanical properties of bone and increase fracture susceptibility independent of BMD (Viguet-Carrin et al, 2006). Assaying of serum or urine samples with Enzyme-Linked Immunosorbent Assay (ELISA) or Radioimmunoassay (RIA) techniques, Procollagen Type 1 N-terminal propeptide (P1NP) and serum C-terminal telopeptide cross-links of type 1 collagen (sCTx) are amongst the newest and clinically relevant biomarkers of bone turnover available (Eastell and Hannon, 2008; Fuller et al, 2008; Gundberg et al, 2002; Malaval et

al, 2008). One cross-sectional study showed that P1NP, a bone formation marker, was significantly elevated in those who regularly exercise greater than 30 min/day (Adami et al, 2008). Furthermore, some studies have shown a potential for exercise to increase P1NP without significant changes in sCTx, a bone resorption marker (Wallace et al, 2000; Landberg et al, 2000; Brahm et al, 1997). In contrast, other exercise studies have found a significant decrease in P1NP post-exercise (Hermann and Seibel, 2008) or significant increases in sCTx post-exercise (Maimoun et al, 2006). Possible reasons for these conflicting findings are: modes of exercise differ (some have used non-weight-bearing exercises including cycling vs weight-bearing exercise including jogging outdoors or on a treadmill); variation in the duration of the exercise (30 min to 3 days of exercise); the intensity of exercise has varied significantly between studies and sometimes has not been well controlled; different ages and genders have been included; and small numbers of subjects have been investigated. To our knowledge, there are no studies which have investigated response of P1NP and sCTx to exercise in premenopausal women with different physical fitness levels.

The purpose of this study was to investigate biomarkers of bone turnover in two groups of premenopausal women, low fitness level versus high fitness level, at rest and their acute response to a single bout of 60 min of aerobic jogging (or brisk walking) to maintain an aerobic intensity of 70-80% maximum heart rate as determined during a VO_2 Max test.

Methods

Subjects

Fifty-one premenopausal women volunteered to participate in the study. Inclusion criteria included females, aged 20-50 years old, having no history of smoking cigarettes and being pre-menopausal as defined by having at least 10 menstrual cycles per year for the last 3 years. Exclusion criteria included current illness; diagnosed osteoporosis, metabolic disease, cardiovascular disease, diabetes or malignancy; currently taking medication known to impair exercise or alter endocrine, hepatic or kidney function; diagnosed respiratory condition known to impair exercise such as asthma; taking over the counter medication including non-steroidal anti-inflammatories; history of an eating disorder; musculoskeletal conditions such as muscular dystrophy or rheumatoid arthritis that would contraindicate exercise participation; consumption of greater than two alcoholic beverages per day (or consumed at least one alcoholic beverage in the last 24 hours prior to the study); any previous fragility fracture or previous fracture within the last 2 years or pregnancy at the time of the study. All procedures were explained to each subject who then signed an informed consent. The study was approved by the Institutional Review Board of Loma Linda University. The study design is outlined in Figure 5 below.

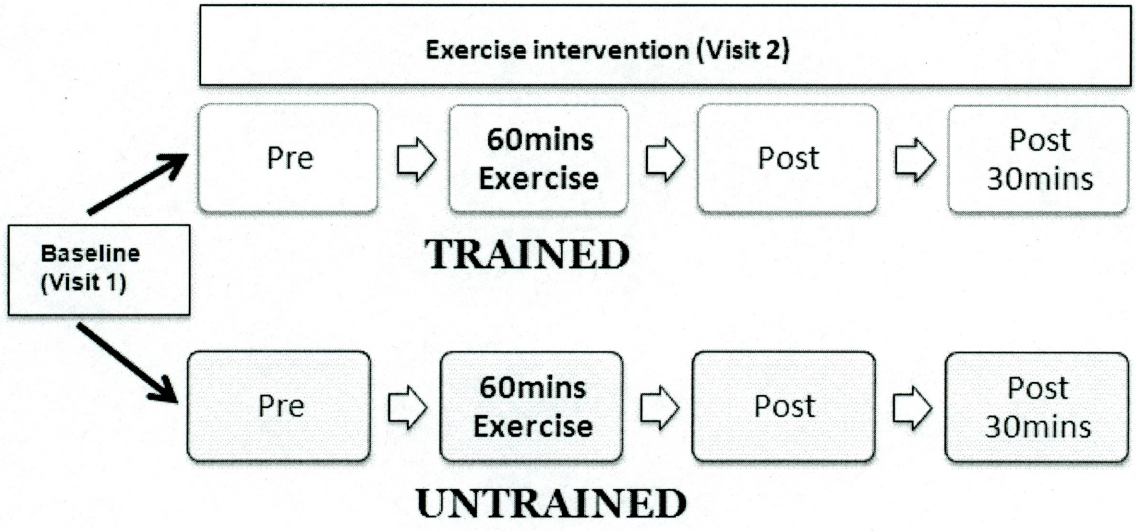

Figure 5. Quasiexperimental multigroup time series design

Fitness and BMD

Within 4 weeks prior to the exercise session, each subject underwent the following tests:

Height, weight, body composition, including body mass index (BMI) and body fat percentage were measured. BMI was calculated as weight (kg)/[height (m)]^2. Body fat percentage and BMD were measured with dual energy x-ray absorptiometry (DXA) (Lunar Prodigy Advance^{TM} 2006, GE Healthcare, Waukesha, WI) (Figure 6). BMD at the lumbar spine and total hip by DXA have been reported in another study as coefficient of variations (CV) 1.9 and 1.6% in post-menopausal women and smaller in children 0.8 and 1.2% respectively (Maimoun et al, 2006). In this study, BMD was measured at the arms, legs, pelvis, spine and total body. T-scores and z-scores were determined; T-score is defined as the number of standard deviations above or below the average of a young adult

at peak bone density; the z-score is the number of standard deviations above or below an average individual of the same age.

Fitness was determined by maximal oxygen uptake (VO_2 Max) measured using the Bruce protocol (American College of Sports Medicine, 2010) on a treadmill and processed with metabolic software (Medical Graphics Corporation, BreezeSuite version 6.3.006, St. Paul, MN) (Figure 7). Heart rate was recorded using and electrocardiogram recorder (Cardio Perfect MD ECG recorder) and ECG software (Welch Allyn Inc, Welch Allyn Cardioperfect version 1.4.2, Skaneateles Falls, NY) (Figure 7). Age-matched fitness was determined by the American College of Sports Medicine guidelines expressed as a percentile between 0-100 (American College of Sports Medicine, 2010).

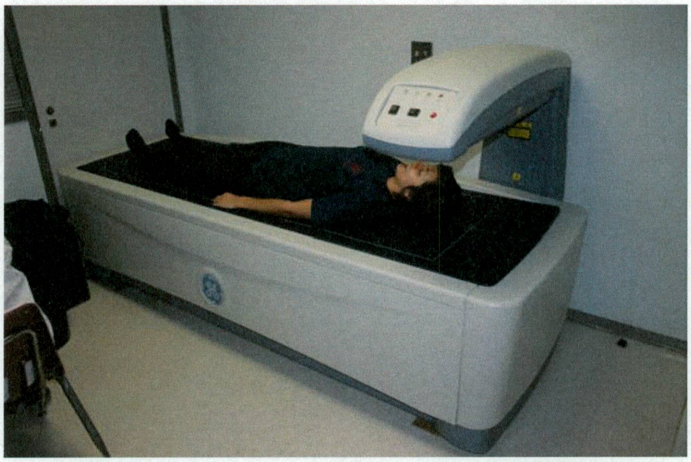

Figure 6. Measurement of BMD by a DXA scan

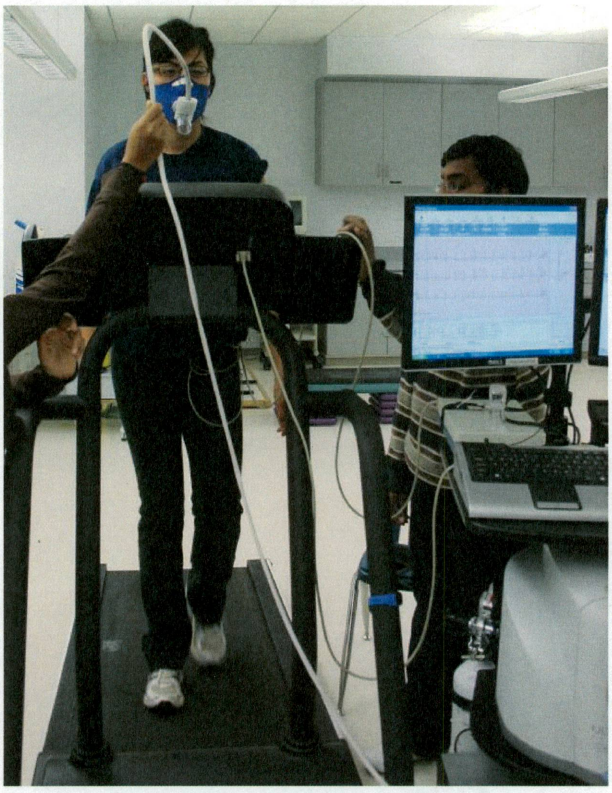

Figure 7. Fitness determined by VO_2 Max using the Bruce protocol.

Questionnaires

To determine dietary intake and physical activity background, two questionnaires were given to all subjects with coded self-addressed envelopes. Dietary intake was assessed using the Block Questionnaire 2005 Food Frequency Questionnaire (FFQ). This includes approximately 110 food items designed to estimate usual and customary intake of a wide array of nutrients and food groups. The food list was developed from NHANES 1999-2002 dietary recall data; the nutrient database was developed from the US Department of Agriculture Food and Nutrient Database for Dietary Studies, version 1.0.

Individual portion size is asked for each food, and pictures are provided to enhance accuracy of quantification (Block Dietary Data Systems, Berkeley, CA). The intraclass correlations for the FFQ range from 0.56 to 0.82 with a median of 0.72 (Hartman et al, 1996). Physical activity background was determined by the Women's Health Initiative Physical Activity Questionnaire (WHIPA) (Meyer et al, 2009). Subjects reported their usual exercise or recreational activity (mild, moderate, strenuous, and walking activities). Responses of physical activity were grouped into three separate intensities (mild, moderate, and strenuous) based a range of MET values associated with the type of activities described. Participants were also asked to report number of hours spent sitting and lying down, including sleep, each day. Test-retest reliability of the WHIPA questionnaire has been determined in large population of women with an intraclass correlation coefficient between 0.55-0.76 (Meyer et al, 2009).

Exercise Protocol

All subjects exercised between 8 and 10am on one day between the months of November and January. Sixty minutes of aerobic jogging or walking (according to the exercise level which was required to maintain the designated aerobic intensity for each individual) was performed on an outdoor track (Figure 8a and 8b). Exercise was set at an aerobic intensity of 70-80% Max heart rate reserve calculated by the Karvonnen Method (American College of Sports Medicine, 2010) utilizing the maximum heart rate determined during their VO_2 Max test. All subjects wore a digital heart rate monitor (Timex, Little Rock, AR) throughout the sixty minutes. Subjects were told to speed up or slow down to maintain their correct heart rate intensity throughout the duration of the

exercise. In addition, subjects wore an accelerometer (GT3X, Actigraph, Pensacola, FL) to calculate number of steps and activity counts of the exercise. Subjects were asked to give their rating of perceived exertion (RPE) post-exercise.

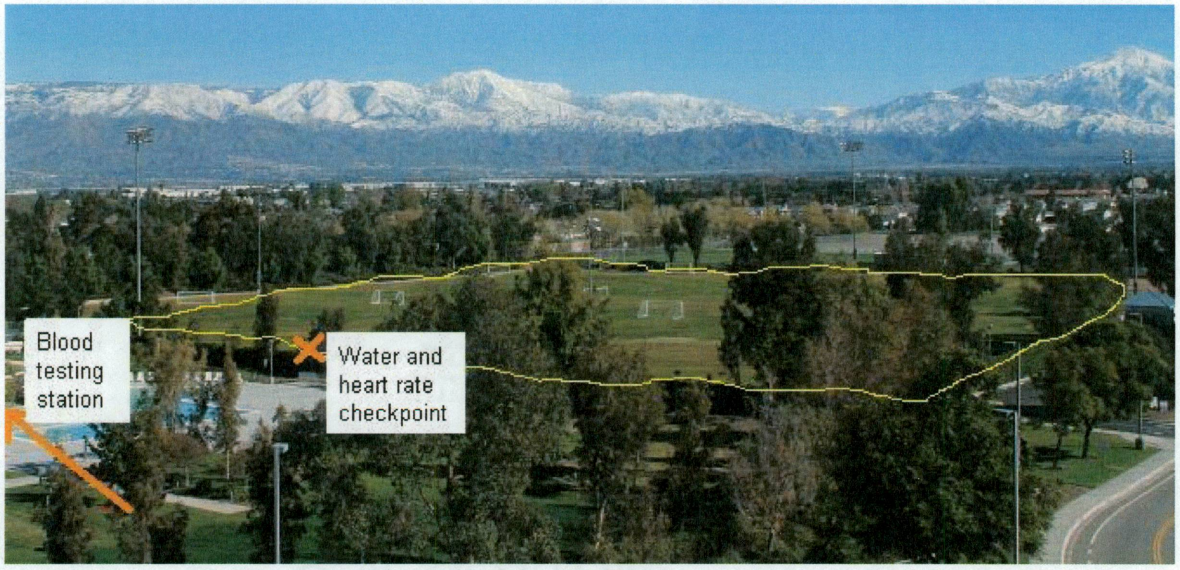

Figure 8a. The exercise was performed on an outdoor track which is 800m per lap. Subjects started and finished at the blood drawing station (indoors).

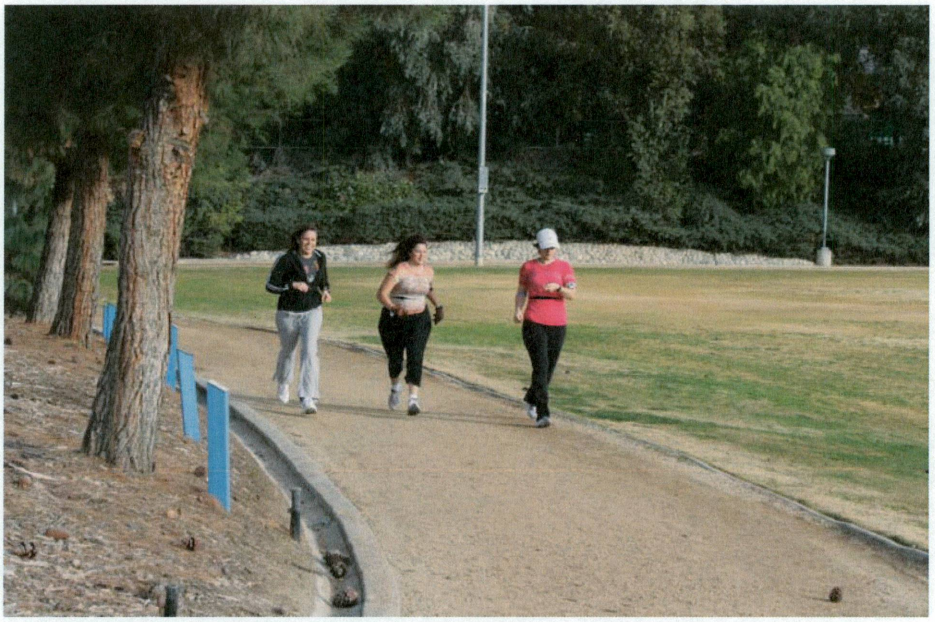

Figure 8b. Subjects during their 60mins exercise intervention.

Blood Testing Sampling and Analysis

Fasting blood samples were collected between 8 am and 10 am at baseline (pre-exercise), immediately post-exercise and 30 min post-exercise. Subjects were permitted to consume water as desired between the pre-exercise blood draw and the 30 min post-exercise blood draw. Blood was drawn by standard venipuncture procedure from an anticubital vein. Blood tubes were placed in a refrigerated centrifuge and spun down for 20 min at 3000 rpm to separate the serum from cells. The serum was then aliquoted into multiple 3.0mL sealed aliquot vials, frozen immediately and stored in a -80deg C non-frost-free freezer. Hematocrit values were used to adjust for changes in plasma volume shifts and subsequent corrections for concentrations of all biomarkers were calculated using the formula of van Beaumont et al, (1973).

17β-Estradiol (intra-assay and inter-assay coefficient of variation (CV) 6.1% and 9.1%, respectively) and intact parathyroid hormone (iPTH) (intra-assay and inter-assay CV 3.2% and 3.6%, respectively) were measured by an automated Enzyme Immunoassay Analyzer (AIA ®360, Tosoh, Grove City, OH). sCTx (intra-assay and inter-assay CV 10.9% and 3.0%, respectively) and 25-OH Vitamin D (intra-assay and inter-assay CV 5.3% and 4.6%, respectively) were measured by ELISA (Immuno Diagnostics Systems, Scottsdale, AZ). P1NP (intra-assay and inter-assay CVs both 9.8%) was measured by RIA technique (Immuno Diagnostics Systems, Scottsdale, AZ). Total serum Ca^{2+} (intra-assay and inter-assay CV 4.8% and 2.1%, respectively) was measured using the Mindray BS-200 analyzer (Point Scientific Inc, Canton, MI).

Statistical Analysis

Data was analyzed using the statistical package SPSS Version 17.0 (SPSS Inc, 2009). Descriptive statistics and frequency distributions were used to summarize the demographics and some characteristics of the subjects. Independent t-tests were used to examine any differences in mean values of the biomarkers of bone turnover between the trained and untrained groups where data was normally distributed. A $\log_{10}$ transformation was performed for bone turnover markers and hormones that were not normally distributed and ANCOVA was used to adjust for group differences in BMI and percentage body fat. Mann-Whitney U-tests were used to test for differences in the mean questionnaire scores and exercise measures between the trained and untrained groups. The level of significance was set at $p < .05$. Effect sizes were calculated to account for group variability. The difference between the pre-exercise mean biomarkers of bone turnover for the trained and untrained groups divided by the standard deviation resulted in an effect size of 1.2. Using a 2-sided significance level of .05 the power was 93%.

Results

From the 51 subjects who started the study, 35 subjects (21 trained, 14 untrained) completed all time points of the study. The reasons for these dropouts were: 4 due to illness, 2 moved from the area, 3 were unable to schedule and 7 had blood drawing difficulties at one or more of the time points. Response rate to the questionnaires was 82.4% (Figure 9).

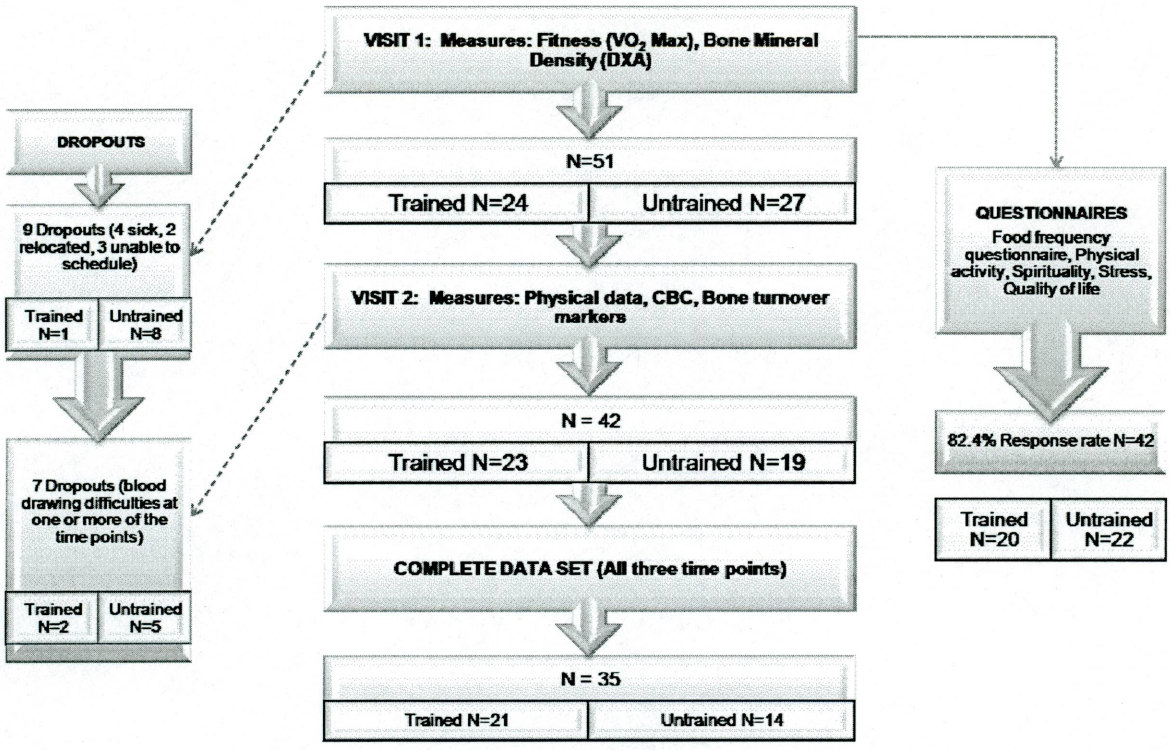

Figure 9. Flowchart of the dropouts throughout the course of the study.

Mean age of the trained group was 32.9±9.9 years and the untrained group was 30.2±8.1 years. There were no significant differences in age or height between groups (Table 1). Mean weight, BMI and body fat percentages were significantly lower in the trained group compared to the untrained group (Table 1).

Fitness and BMD

VO_2 Max and age-matched fitness by percentile ranking were significantly higher in the trained compared to the untrained group (42.2±5.3 vs 27.9±3.3ml/kg/min, $p<.001$, and 79.7±16.7 vs 13.3±8.5%, $p<.001$). BMD was adjusted for group differences in BMI and percentage body fat. There were no significant differences between the groups in

BMD at the arms, legs, pelvis, spine or total body BMD, z-score, age-matched z-score, T-score or age-matched T-score between the groups (Table 1).

Table 1. Mean (SD) of demographics, bone density and fitness measures for the trained and untrained groups (n=35)

Variable	**Trained ($n_1 = 21$)**	**Untrained ($n_2 = 14$)**	***p* Value**[+]
Age (years)	32. 9(9.9)	30.2(8.1)	NS
Height (cm)	163.3(5.8)	166.7(6.3)	NS
Weight (kg)	59.5(5.9)	70.5(12.7)	**.01**
Body mass index[*]	22.3(2.3)	25.4(4.4)	**.04**
Body fat (%)	26.4(5.8)	38.8(6.9)	**<.001**
VO_2 Max (mL/kg/min)	42.2(5.3)	27.9(3.3)	**<.001**
Age-Matched Fitness	79.7(16.7)	13.3(8.5)	**<.001**
Arms BMD (g/cm^2)[**]	0.853(0.055)	0.935(0.095)	NS
Legs BMD (g/cm^2)	1.261(0.110)	1.278(0.102)	NS
Pelvis BMD (g/cm^2)	1.167(0.112)	1.195(0.084)	NS
Spine BMD (g/cm^2)	1.035(0.112)	1.090(0.070)	NS
Total BMD (g/cm^2)	1.167(0.077)	1.197(0.018)	NS
z-score	0.762(0.893)	0.736(0.925)	NS
Age-matched z-score	105.5(6.3)	105.4(6.5)	NS
T-score	0.562(0.923)	0.986(0.795)	NS
Age-matched T-score	104.0(6.6)	107.0(5.6)	NS

[+] *p* Values were calculated using Independent t-tests
[*] Body mass index calculated as weight(kg)/[height(m)]^2
[**] BMD=Bone mineral density. BMD was adjusted for differences in BMI and % body fat

Questionnaires

There were no significant differences in calcium intake from foods or vitamins, consumption of fruits and vegetables, protein, caffeine or sodium intake between the groups ($p>.05$) (Table 2).

Table 2. Mean (SE) of dietary intake measures for the trained and untrained groups (n=31)

Variable	**Trained ($n_1 = 19$)**	**Untrained ($n_2 = 14$)**	***p* Value**[+]
Dietary preference	Vegetarian n=18 Non-Vegetarian n=1	Vegetarian n=10 Non-Vegetarian n=2	
Calcium intake/day from foods (mg)	534(63)	401(69)	.19
Calcium intake/day from vitamins (mg)	197(60)	169(87)	NS
Total calcium intake/day (mg)	731(98)	570(124)	.21
No. of Fruits/day	2.0(0.3)	2.1(0.5)	NS
No. of Vegetables/day	2.1(0.2)	1.6(0.9)	.17
Protein intake/day (g)	41.0(5.4)	40.6(5.7)	NS
Caffeine intake/day (mg)	115(26)	102(23)	NS
Sodium intake/day (mg)	1120(137)	990(126)	NS

[+]*p* Values were calculated using Mann-Whitney U-tests

For the WHIPA questionnaire, mild physical activity, number of hours sitting, number of hours sleeping and total sedentary activity were not significantly different between the two groups. Mean moderate physical activity in the trained group was nearly twice the untrained group (8.4±1.4 vs 4.7±1.2METS/hour/week, p=.049), mean strenuous

physical activity was significantly higher in the trained group compared to the untrained group (6.2±0.4 vs 3.6±0.9METS/hour/week, p=.04), mean moderate to strenuous physical activity was significantly higher in the trained group compared to the untrained group (14.7±1.4 vs 8.3±1.8METS/hour/week, p=.01), and total exercise and recreational activity was significantly higher in the trained group compared to the untrained group (16.8±1.8 vs 10.1±2.2METS/hour/week, p=.01) (Table 3).

Table 3. Mean (SE) of questionnaire measures for the trained and untrained groups (n=35)

Variable	**Trained ($n_1 = 21$)**	**Untrained ($n_2 = 14$)**	***p* Value[+]**
Mild Physical Activity (METS/hr/week)	2.1 (0.7)	1.8 (0.7)	NS
Moderate Physical Activity (METS/hr/week)	8.4 (1.4)	4.7(1.2)	**.05**
Strenuous Physical Activity (METS/hr/week)	6.2(0.4)	3.6(0.9)	**.04**
Walking and Recreational Physical Activity (METS/hr/week)	9.7(1.7)	8.3(2.1)	NS
Moderate to Strenuous Physical Activity (METS/hr/week)	14.7 (1.4)	8.3 (1.8)	**.01**
Total Exercise and Recreational Exposure (METS/hr/week)	16.8(1.8)	10.1(2.2)	**.01**
Sitting (hrs/week)	45.7(5.0)	47.4(6.0)	NS
Sleeping (hrs/week)	50.0(4.3)	57.3(2.7)	NS
Total Sedentary Activity (hrs/week)	98.4(6.7)	104.7(6.3)	NS

[+]*p* Values were calculated using Mann-Whitney U-tests

Menstrual Status and 17-β Estradiol

The proportion of menstrual states was similar in the two groups. Mean 17β-Estradiol was not significantly different between the trained and untrained group (94.6±54.6 vs 90.7±66.8pg/ml, $p>.05$). Frequency distribution of menstrual status between groups was similar (trained: 38% luteal, 43% follicular, 5% 1 day before lutenizing hormone(LH) surge, 14% 2 days after LH surge; untrained 43% luteal, 50% follicular, 7% 1 day before LH surge, $p>.05$).

Exercise Measures

Mean RPE was significantly lower in the trained group compared with the untrained group (10.9±0.3 vs 12.1±0.6, $p=.003$). Distance and total number of steps during the 60 min of aerobic exercise were significantly higher in the trained group compared to the untrained group (9532±317 vs 6773±247m, $p<.001$, and 10138±105 vs 8596±260 steps, $p<.001$). Mean activity counts was also significantly higher in the trained group compared to the untrained group (1799±87 vs 1524±52, $p=.01$).

Plasma Volume

Mean hematocrit was not significantly different between the trained and untrained groups at any of the time points. In the trained group, hematocrit significantly increased from pre-exercise to post-exercise (40.6±0.7 vs 41.4±0.6g/dL, $p=.004$). In the untrained group, hematocrit significantly increased from pre-exercise to post-exercise (39.1±0.9 vs 40.1±0.8g/dL, $p=.03$) and significantly decreased from post-exercise to 30 min post-

exercise (39.4±0.7 vs 39.1±0.9g/dL, p=.03). All CBC measures are listed in Tables 1, 2 and 3 in Appendix A.

Hormones and Markers of Bone Turnover

The following concentrations of hormones and biomarkers of bone turnover were adjusted for changes in plasma volume shifts. The results for sCTx, P1NP, total Ca^{2+}, iPTH, 25-OH Vitamin D and Cortisol are displayed in Figure 10 and Table 5, 6 and 7. Although not significantly different, mean sCTx there was a trend towards higher levels in the trained group compared to the untrained group at all time points (pre-exercise 607±59 vs 505±75 ng/L, p=.21, post-exercise 645±59 vs 448±77 ng/L, p=.05 and 30 min post-exercise 597±58 vs 438±66 ng/L, p=.06). In the trained group, mean sCTx significantly decreased from pre-exercise to 30 min post-exercise (607±59 vs 597±58ng/L, p=.04). In the untrained group, mean sCTx was not significantly different at any of the time points (p>.05).

Mean P1NP was significantly higher in the trained group compared to the untrained group at post-exercise (76.0±5.0 vs 47.2±5.3µg/L, p<.001) and 30 min post-exercise (57.4±3.7 vs 42.6±5.0µg/L, p=.01). Mean P1NP significantly increased from pre-exercise to post-exercise in both groups (trained 58.8±3.7 to 76.0±5.0µg/L, p<.001, and untrained 40.0±4.0 to 47.2±5.3µg/L, p=.001) and at 30 min post-exercise, mean P1NP returned to pre-exercise levels in both groups.

Mean Ca^{2+} was not significantly different between the trained and untrained groups at any of the time points. In both groups there was an increase in mean Ca^{2+} from pre-exercise to post-exercise but this difference was only significant in the untrained

group (10.8±0.1 vs 11.1±0.2mg/dL, p=.001). In both groups there was a decrease in mean Ca^{2+} from post-exercise to 30 min post-exercise but this difference was only significant in the trained group (11.6±0.2 vs 10.8±0.2mg/dL, p<.001).

Although not significant, there was a trend toward lower mean iPTH levels in the trained group compared to the untrained group at all time points. Mean iPTH levels in both groups significantly increased from pre-exercise to post-exercise (trained 40.7±2.1 vs 73.2±5.4pg/mL, p<.001 and untrained 49.0±6.3 vs 78.3±12.8pg/mL, p<.001), then significantly decreased from post-exercise to 30 min post-exercise (trained 73.2±5.4 vs 40.5±1.8pg/mL, p<.001 and untrained 78.3±12.8 vs 45.9±6.5pg/mL, p=.002).
Mean 25-OH Vitamin D was significantly higher in the trained group compared to the untrained group at pre-exercise (93.2±12.0 vs 54.5±6.3nmol/L, p=.01), post-exercise (101.2±12.7 vs 63.3±7.8nmol/L, p=.01) and 30 min post-exercise (92.2±11.4 vs 61.2±8.2nmol/L, p=.02). Mean 25-OH Vitamin D significantly increased from pre-exercise to post-exercise in both groups (trained 93.2±12.0 vs 101.2±12.7nmol/L, p=.01 and untrained 54.5±6.3 vs 63.3±7.8nmol/L, p=.02). In the untrained group, 25-OH Vitamin D significantly increased from pre-exercise to 30 min post-exercise (61.2±8.2 vs 54.5±6.3nmol/L, p=.02). In the trained group, mean 25-OH Vitamin D pre-exercise and 30 min post-exercise were not significantly different (p>.05).

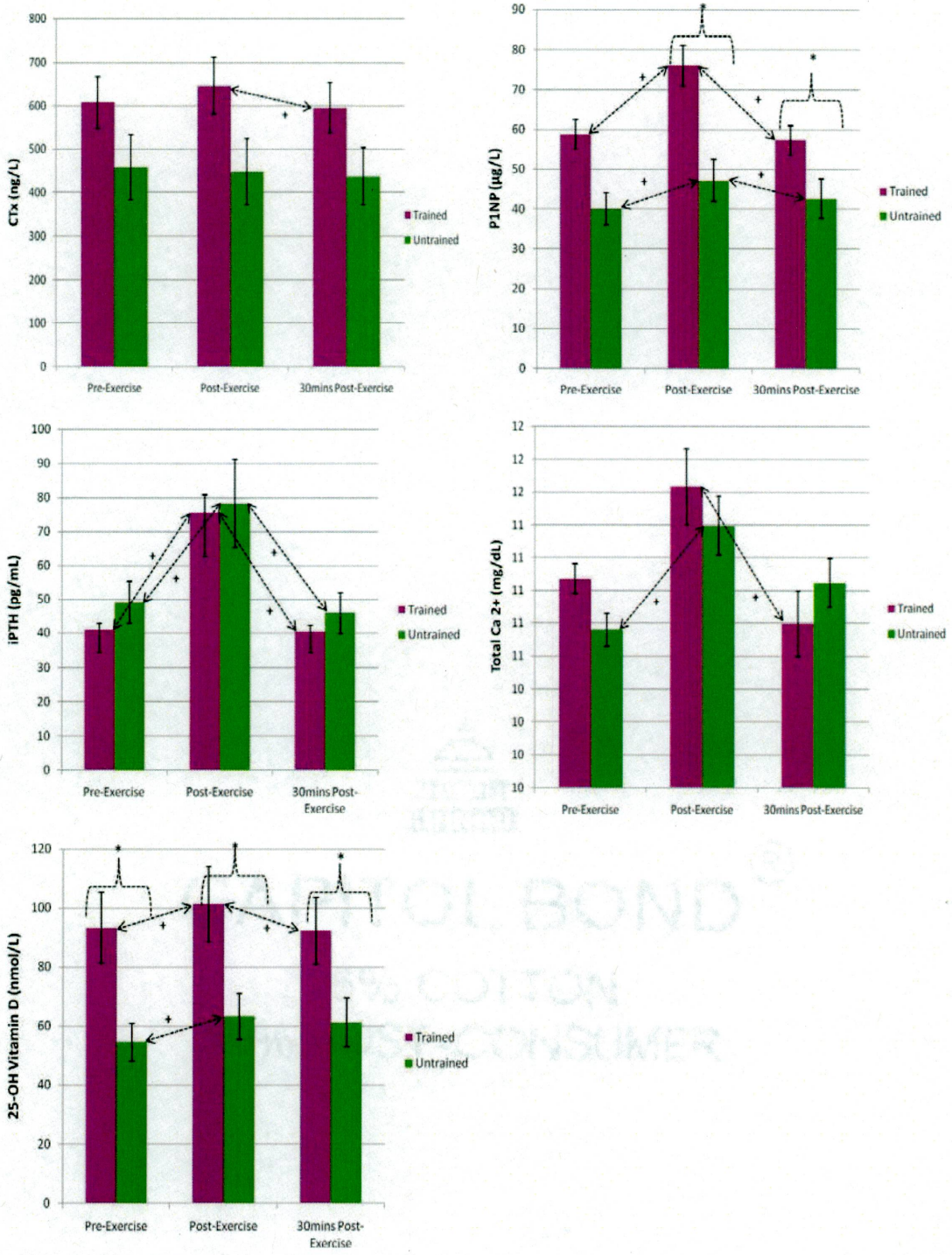

Figure 10. Mean (SE) concentrations of biomarkers of bone turnover in the trained (purple) and untrained (green) groups. All concentrations have been corrected for changes in plasma volume shifts. Data has been corrected for BMI and % body fat differences

$^{+}$ within group difference $p<.05$

* between group difference $p<.05$

Table 4. Mean (SE) of biomarkers of bone turnover before, after and 30mins after exercise in the trained group (n=21)

Marker	Pre-exercise	Post-exercise	30mins Post-exercise	*p* Value[+]	*p* Value[*] (pre vs. post)	*p* Value[*] (post vs. 30mins post)	*p* Value[*] (pre vs. 30mins post)
CTx (ng/L)	607(59)	645(65)	597(58)	.11	.08	**.04**	NS
P1NP(µg/L)	58.8(3.7)	76.0(5.0)	57.4(3.7)	**<.001**	**<.001**	**<.001**	NS
Ca^{2+}(mL/dL)	11.1(0.1)	11.6(0.2)	10.8(0.2)	**.001**	.08	**<.001**	.15
iPTH (pg/mL)	40.7(2.1)	73.2(5.4)	40.5(1.8)	**<.001**	**<.001**	**<.001**	NS
25-OH Vitamin D (nmol/L)	93.2(12.0)	101.2(12.7)	92.2(11.4)	**<.001**	**.01**	**<.001**	NS

[+] *p* Values were calculated using one-way repeated measures ANOVA with Log_{10} transformation
[*] *p* Values were calculated using Bonferroni post-hoc testing

Table 5. Mean (SE) of biomarkers of bone turnover before, after and 30mins after exercise in the untrained group (n=14)

Marker	Pre-exercise	Post-exercise	30mins Post-exercise	*p* Value[+] [+]	*p* Value[*] (pre vs. post)	*p* Value[*] (post vs. 30mins post)	*p* Value[*] (pre vs. 30mins post)
CTx (ng/L)	505(75)	448(77)	438(66)	.17	NS	NS	NS
P1NP(µg/L)	40.0(4.0)	47.2(5.3)	42.6(5.0)	**.002**	**.001**	**.03**	NS
Ca^{2+}(mL/dL)	10.8(0.1)	11.1(0.2)	11.1(0.1)	**.01**	**.01**	NS	NS
iPTH (pg/mL)	49.0(6.3)	78.3(12.8)	45.9(6.1)	**<.001**	**.002**	**<.001**	NS
25-OH Vitamin D (nmol/L)	54.5(6.3)	63.3(7.8)	61.2(8.2)	**.03**	**.02**	NS	NS

[+] *p* Values were calculated using one-way repeated measures ANOVA with Log_{10} transformation
[*] *p* Values were calculated using Bonferroni post-hoc testing

Table 6. Mean (SE) of biomarkers of bone turnover of the trained and untrained groups before, after and 30mins after exercise (n=35)

Marker	**Pre-exercise** Trained (n_1=21)	**Pre-exercise** Untrained (n_2=14)	***p* Value**[+]	**Post-exercise** Trained (n_1=21)	**Post-exercise** Untrained (n_2=14)	***p* Value**[+]	**30mins Post-exercise** Trained	**30mins Post-exercise** Untrained	***p* Value**[+]
CTx(ng/L)	607 (59)	505 (75)	NS	645 (65)	448(77)	NS	597(58)	438(66)	NS
P1NP(µg/L)	58.8(3.7)	40.0(4.0)	.10	76.0(5.0)	47.2(5.3)	**<.001**	57.4(3.7)	42.6(5.0)	**.01**
Ca^{2+}(mL/dL)	11.1(0.1)	10.8(0.1)	NS	11.6(0.2)	11.1(0.2)	NS	10.8(0.2)	11.1(0.1)	NS
iPTH (pg/mL)	40.7(2.1)	49.0(6.3)	NS	73.2(5.4)	78.3(12.8)	NS	40.5(1.8)	45.9(6.1)	NS
25-OH Vitamin D (nmol/L)	93.2(12.0)	54.5(6.3)	**.01**	101.2(12.7)	63.3(7.8)	**.01**	92.2(11.4)	61.2(8.2)	**.02**

[+]*p* Values were calculated using Independent t-tests with Log_{10} transformation adjusted for differences in BMI and % body fat between groups

Discussion

Higher levels of P1NP and sCTx were observed in the trained group compared with the untrained group across all three time points inspite of no significant age difference between the groups. A potential reason could be that the fitness of the trained group provided a more favorable bone turnover profile similar to that of younger premenopausal women. Higher P1NP levels in individuals reporting more physical activity has been reported in a large cross-sectional study (Adami et al, 2008). This finding suggests that fitness could impact the bone turnover profile of premenopausal women delaying the age-related progression towards a decline of bone turnover prior to menopause.

Mean P1NP was elevated post-exercise in both the trained and untrained groups, yet this difference reached statistical significance only in the trained group. Increases in P1NP or Procollagen Type 1 C-terminal propeptide (P1CP) post-exercise have been reported in some studies (Brahm et al, 1997; Evans et al, 2008) but not in others (Thorsen et al, 1997; Toson et al, 2006). This could be due to differences in exercise intervention and blood collection methodology. In this study, it appears that the mechanical loads placed on bone during the aerobic exercise may have stimulated bone formation in both groups, but may be more osteogenic in the trained group.

Mean sCTx trended to be higher post-exercise in the trained group compared to the untrained group but did not reach the level of significance. This slight elevation could have reflected the initiation of osteoclastic activity and subsequent bone formation as observed by a significant increase in P1NP post-exercise in the trained group. Mean sCTx was not significantly different at 30 min post-exercise compared with pre-exercise

in both groups. There is limited evidence investigating sCTx with response to exercise in premenopausal women. One study found an initial increase after 2 months but a return to baseline at 4 months with a military exercise intervention (Evans et al, 2008).

The Ca^{2+} and iPTH findings were similar to other exercise studies investigating premenopausal women with an increase post-exercise (Brahm et al, 1997; Thorsen et al, 1997; Toson et al, 2006; Rudberg et al, 2000) and returning to baseline by 30 min post-exercise (Brahm et al, 1997; Rudberg et al, 2000). These results were similar for both the trained and untrained groups. Mean iPTH levels were lower at baseline in the trained group compared to the untrained group and this may reflect the potential for exercise training to create a new set point for iPTH adjusting for increased Ca^{2+} demands associated with an increased metabolism. In a previous study, lower iPTH levels were observed after 2 months of exercise training yet iPTH levels returned to baseline at 4 months (Evans et al, 2008). Another possibility for the difference in iPTH between the groups is that elevated iPTH in the untrained group could be due to inadequate intake of calcium. Daily calcium intake for both groups was lower than the recommended 1000 mg/day, yet the calcium intake for the trained group was 725 mg/day compared to the untrained group which was 490 mg/day.

We found significantly higher 25-OH Vitamin D levels in the trained group compared to the untrained group. This is contrary to another study which did not find any differences in Vitamin D between individuals that reported exercising and those who did not (Glover et al, 2008). The 25-OH Vitamin D values for the untrained group in this study were comparable another study (De Papp et al, 2007) yet they were well below the recommended level of 75 nmol/L suggesting that a large majority of premenopausal

women may be either insufficient or deficient in Vitamin D (NOF, 2010). However, 25-OH Vitamin D levels in both groups significantly increased from pre-exercise to post-exercise illustrating the efficacious role of outdoor aerobic exercise on Vitamin D concentrations.

In this study, similar to a previous meta-analysis of exercise in premenopausal women (Kelley and Kelley, 2004), total and regional BMD measures were not significantly different between the trained and untrained groups, yet there were several differences in the biomarker for bone turnover profile of these groups. This suggests and supports the need to continue to evaluate biomarkers of bone turnover as a potential predictive and assessment measure for osteoporosis risk in premenopausal women.

Strengths of this study include the methodological rigor of the exercise intervention and blood collection. Parameters of the exercise intervention have been clearly defined by several measures including intensity (RPE, % relative HR intensity, activity counts), duration (distance, number of steps) and time. The exercise intervention was based on current guidelines for bone health in healthy adults (Physical Activity Guidelines Advisory Committee, 2008) and applicable as all subjects were able to maintain their aerobic heart rate intensity throughout the 60 min of exercise regardless of their level of fitness. Blood collection methods were controlled to minimize any variability of the biomarkers which included subject fasting and circadian variation with greatest sensitivity of sCTx between 8 and 10 am (Hermann and Seibel, 2008; Qvist et al, 2002).

Conclusion

Despite that there were no differences in mean BMD between trained and untrained premenopausal women, there were significant differences in markers of bone turnover both at baseline and with response to aerobic exercise. The implications of these findings include the potential use of these biomarkers to evaluate individuals' bone remodeling responses at rest and to exercise which may result in earlier identification of risk factors for osteoporosis. This study supports the efficacy of weight-bearing aerobic exercise in promoting bone remodeling at all fitness levels, but with greater responses observed in individuals who are already maintaining higher fitness levels.

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CHAPTER FOUR

PUBLISHABLE PAPER II

FITNESS (VO_2 MAX) IN A PREDICTION MODEL OF BONE FORMATION P1NP IN PREMENOPAUSAL WOMEN

Abstract

Procollagen type 1 N-terminal propeptide (P1NP) has shown to be a significant biomarker for bone formation. The purpose of this study was to investigate the relationship of fitness with P1NP and markers of bone turnover in premenopausal women. Forty-two premenopausal women, mean age 31.6±8.8 years, underwent fitness and bone mineral density (BMD) testing and a blood draw for biomarkers of bone turnover. Serum was analyzed for P1NP, biomarker for bone resorption, C-telopeptide cross-links of type 1 collagen (sCTx) and endocrine regulation, 17-β Estradiol, 25-OH Vitamin D, calcium (Ca^{2+}), and intact parathyroid hormone (iPTH). Data was analyzed using Spearman's correlation coefficient and stepwise multiple regression. P1NP significantly correlated with VO_2 Max (r =.47, p=.002), sCTx (r =.72, p<.001), 25-OH Vitamin D (r =.42, p=.01), Ca^{2+} (r =.35, p=.02), age (r =-.36, p=.03), % body fat (r =-.34, p=.03) and spine BMD (r =-.39, p=.01). Fifty-eight percent of the variability in P1NP was explained by its relationship to VO_2 Max and sCTx (p=.02). This unique prediction model for bone formation P1NP includes VO_2 Max and sCTx. The finding demonstrates that fitness has a significant role in evaluating and predicting bone remodeling in

premenopausal women. Determining fitness in this population could facilitate the identification of risk factors for osteoporosis by providing insight into the rate of bone remodeling.

Introduction

Osteoporosis is a disease which affects 55 percent of people over the age of 50 and 80 percent of those diagnosed with osteoporosis are women, (National Osteoporosis Foundation, 2010). While it is understood that in order to optimize bone health adults need to engage in regular weight-bearing physical activity (Physical Activity Guidelines Advisory Committee, 2008), there are few objective fitness measures relating to osteoporosis risk. In comparison to measurement of bone mineral density (BMD), blood testing for markers of bone turnover may be more practical and clinically relevant in determining the collagen remodeling properties of bone.

The N-terminal extension of procollagen (P1NP) and a degradation product of the C-telopeptide of α1 chain of type 1 collagen (CTx) mature type 1 collagen have been shown to be significant biomarkers in the investigation of growth and bone metabolism (Eastell and Hannon, 2008) and some studies have started to propose normal reference ranges for these markers in healthy young premenopausal women (Adami et al, 2008; Adami et al, 2008; Brahm et al, 1997; Glover et al, 2008). However, many studies have not included measures of physical activity participation (Glover et al, 2008) or have relied on self-reported physical questionnaires (Adami et al, 2008, Adami et al, 2008, Brahm et al, 1997). There is some evidence that fitness, as determined by VO_2 max, may be related to markers of bone turnover (Evans et al, 2008, Brahm et al, 1997). Evans et al,

(2008) investigated the relationship of VO_2 Max and P1NP, CTx, Ca^{2+}, iPTH, and 25-OH Vitamin D in female military recruits. CTx significantly positively correlated with VO_2 Max, height and lean body mass and negatively correlated with fat mass, % body fat and 25-OH Vitamin D. VO_2 Max, however, was not significant enough to enter into a regression model and there was no significant relationship between VO_2 Max and P1NP ($p>.05$) (Evans et al, 2008).

The purpose of this study was to investigate the relationship of fitness, as determined by VO_2 Max with P1NP, sCTx, 25-OH Vitamin D and other anthropometric variables in premenopausal women.

Methods

Subjects

Forty-two premenopausal women volunteered to participate in the study. Inclusion criteria included females, aged 20-50 years old, having no history of smoking cigarettes and being premenopausal as defined by having at least 10 menstrual cycles per year for the last 3 years. Exclusion criteria included current illness; diagnosed osteoporosis, metabolic disease, cardiovascular disease, diabetes or malignancy; currently taking medication known to impair exercise or alter endocrine, hepatic or kidney function; diagnosed respiratory condition known to impair exercise such as asthma; taking over the counter medication including non-steroidal anti-inflammatories; history of an eating disorder; musculoskeletal conditions such as muscular dystrophy or rheumatoid arthritis; consumption of greater than two alcoholic beverages per day (or consumed at least one alcoholic beverage in the last 24 hours prior to the study); any

previous fragility fracture or previous fracture within the last 2 years or pregnancy at the time of the study. All procedures were explained to each subject who then signed an informed consent. The study was approved by the Institutional Review Board of Loma Linda University.

Anthropometric, Fitness and BMD

Height, weight, body composition, including body mass index (BMI) and body fat percentage were measured. BMI was calculated as weight (kg)/[height (m)]^2. Body fat percentage and BMD were measured with dual energy x-ray absorptiometry (DXA) (Lunar Prodigy Advance^{TM} 2006, GE Healthcare, Waukesha, WI). BMD at the lumbar spine and total hip by DXA have been reported in another study as coefficient of variations (CV) 1.9 and 1.6% in post-menopausal women and smaller in children 0.8 and 1.2% respectively (Maimoun et al, 2006). In this study, BMD was measured at the arms, legs, pelvis, spine and total body. T-scores and z-scores were determined; T-score was defined as the number of standard deviations above or below the average of a young adult at peak bone density; the z-score was the number of standard deviations above or below an average individual of the same age.

Fitness was determined by maximal oxygen uptake (VO_2 Max) measured using the Bruce protocol (American College of Sports Medicine, 2010) on a treadmill and processed with metabolic software (Medical Graphics Corporation, BreezeSuite version 6.3.006, St. Paul, MN). Heart rate was recorded using and electrocardiogram recorder (Cardio Perfect MD ECG recorder) and ECG software (Welch Allyn Inc, Welch Allyn Cardioperfect version 1.4.2, Skaneateles Falls, NY). Age-matched fitness was

determined by the American College of Sports Medicine guidelines expressed as a percentile between 0-100 (American College of Sports Medicine).

Blood Testing Sampling and Analysis

Fasting blood samples were collected between 8 am and 9 am on a separate day, within 4 weeks of the fitness and BMD testing. Blood was drawn by standard venipuncture procedure from an anticubital vein. Blood tubes were placed in a refrigerated centrifuge and spun down for 20 min at 3000 rpm to separate the serum from cells. The serum was then aliquoted into multiple 3.0mL sealed aliquot vials, frozen immediately and stored in a -80deg C non-frost-free freezer. 17β-Estradiol (intra-assay and inter-assay coefficient of variation (CV) 6.1% and 9.1%, respectively) and intact parathyroid hormone (iPTH) (intra-assay and inter-assay CV 3.2% and 3.6%, respectively) were measured by an automated Enzyme Immunoassay Analyzer (AIA ®360, Tosoh, Grove City, OH). Serum CTx (intra-assay and inter-assay CV 10.9% and 3.0%, respectively) and 25-OH Vitamin D (intra-assay and inter-assay CV 5.3% and 4.6%, respectively) were measured by ELISA (Immuno Diagnostics Systems, Scottsdale, AZ). P1NP (intra-assay and inter-assay CVs both 9.8%) was measured by RIA technique (Immuno Diagnostics Systems, Scottsdale, AZ). Total serum Ca^{2+} (intra-assay and inter-assay CV 4.8% and 2.1%, respectively) was measured using the Mindray BS-200 analyzer (Point Scientific Inc, Canton, MI).

Statistical Analysis

Data was analyzed using the statistical package SPSS Version 17.0 (SPSS Inc, 2009). Descriptive statistics and frequency distributions were used to summarize the demographics and some characteristics of the subjects. We examined the relationship between the VO_2 Max, sCTx, 25-OH Vitamin D, BMD and anthropometric variables (age, height, weight, BMI, % body fat) with the biomarker of bone formation, P1NP, using Spearman rank-order correlation. To identify the biomarkers that affect P1NP, stepwise multiple regression was used with P1NP as the dependent variable and VO_2 Max, anthropometric measures and BMD as predictor variables. All analyses were conducted using a significance level of $\alpha = .05$.

Results

Mean age of the subjects was 31.6±8.8 years and mean VO_2 Max was 36.0±8.4 mL/kg/min. Mean height, weight, BMI, percentage body fat, total and regional BMD, z-score and T-score are listed in Table 8. Mean 17β-Estradiol was 98.5±60.1 pg/mL. Frequency distribution of menstrual status was 49% luteal, 36% follicular, 5% one day before lutenizing hormone (LH) surge and 10% two days after LH surge.

Table 7 Descriptives of demographics, bone mineral density and fitness measures for the subjects (n=42)

Variable	Mean (SD)	Median	Range
Age (years)	31.6(8.8)	29.9	20.0-49.8
Height (cm)	165.2(5.9)	165.1	148.6-178.0
Weight (kg)	64.2(10.2)	61.6	48.0-94.1
Body mass index*	23.5(3.5)	22.5	17.6-35.6
Body fat (%)	31.8(8.9)	32.7	16.6-51.1
VO_2 Max (mL/kg/min)	36.0(8.4)	35.4	21.7-54.1
Arms BMD (g/cm^2)**	0.884(0.077)	0.877	0.734-1.191
Legs BMD (g/cm^2)	1.273(0.106)	1.283	1.035-1.468
Pelvis BMD (g/cm^2)	1.167(0.126)	1.184	0.646-1.401
Spine BMD (g/cm^2)	1.060(0.093)	1.057	0.855-1.234
Total BMD (g/cm^2)	1.179(0.070)	1.185	0.993-1.317
z-score	0.710(0.885)	0.850	-1.0-2.2
T-score	0.719(0.842)	0.800	-1.6-2.4

*Body mass index calculated as weight(kg)/[height(m)]^2
**BMD=Bone mineral density.

Determination of the Prediction Model

Correlations with P1NP

Mean values for the biomarkers of bone turnover are displayed in Table 2. Correlations between age, % body fat, spine BMD, VO_2 Max and biomarkers of bone turnover are shown in Table 3.

P1NP significantly correlated with seven variables: VO_2 Max (r =.47, p=.002), sCTx (r =.72, p<.001), 25-OH Vitamin D (r =.42, p=.01), Ca^{2+} (r =.35, p=.02), age (r =-.36, p=.03), % body fat (r =-.34, p=.03) and spine BMD (r =-.39, p=.01). The linear relationships between VO_2 Max and P1NP and sCTx are shown in Figure 1. When adjusted for age and BMI, P1NP remained significantly correlated with VO_2 Max (r =.39, p=.01) and sCTx (r =.68, p<.001).

Stepwise Multiple Regression model with P1NP

Fifty-eight percent of the variability in P1NP was explained by two variables: VO_2 Max and sCTx (R^2 = 58%, p=.02). sCTx accounted for 51% and VO_2 Max accounted for 7% of the overall variability in P1NP.

$$\hat{P1NP} = 5.28 + 0.44sCTx + 0.60VO_2 \text{ Max}$$

Equation 1. Prediction model

Table 8. Descriptives of biomarkers of bone turnover for the subjects (n=42)

Variable	Mean (SD)	Median	Range
P1NP (µg/dL)	51.9(18.5)	45.5	21.5-93.9
sCTx (ng/L)	567.7(266.3)	523.5	166-1387
25-OH Vitamin D (nmol/L)	77.0(45.9)	62.7	33.8-256.0
iPTH (pg/mL)	44.8(16.5)	43.2	23.8-93.7
Ca^{2+}(mL/dL)	10.9(0.4)	10.9	10.3-11.7

Table 9. Correlations[+] between subjects' characteristics and biomarkers of bone turnover (n=42)

Variable	1. P1NP (µg/dL)	2. VO_2 Max (mL/kg/min)	3. sCTx (ng/L)	4. 25-OH Vitamin D (nmol/L)	5. iPTH (pg/mL)	6. Ca^{2+} (mL/dL)	7. Age (years)	8. % Body fat	9. Spine BMD (g/cm^2)
1. P1NP (µg/dL)									
2. VO_2 Max (mL/kg/min)	.47**								
3. sCTx (ng/L)	.72**	.34*							
4. 25-OH Vitamin D (nmol/L)	.42**	.45**	.33*						
5. iPTH (pg/mL)	-.16	-.31*	-.19	-.16					
6. Ca^{2+}(mL/dL)	.35*	.53**	.25	.31*	-47**				
7. Age (years)	-.35*	-.13	-.47**	.18	.30	-.162			
8. % Body fat	-.34*	-.86**	-.24	-.38*	.18	-.47**	-.05		
9. Spine BMD (g/cm^2)	-.39*	-.33*	-.35*	-.24	.06	.002	.23	.35*	

** Correlation is significant at the .01 level (2-tailed)

* Correlation is significant at the .05 level (2-tailed)

[+] Correlations were obtained using Spearman's Ranked correlation

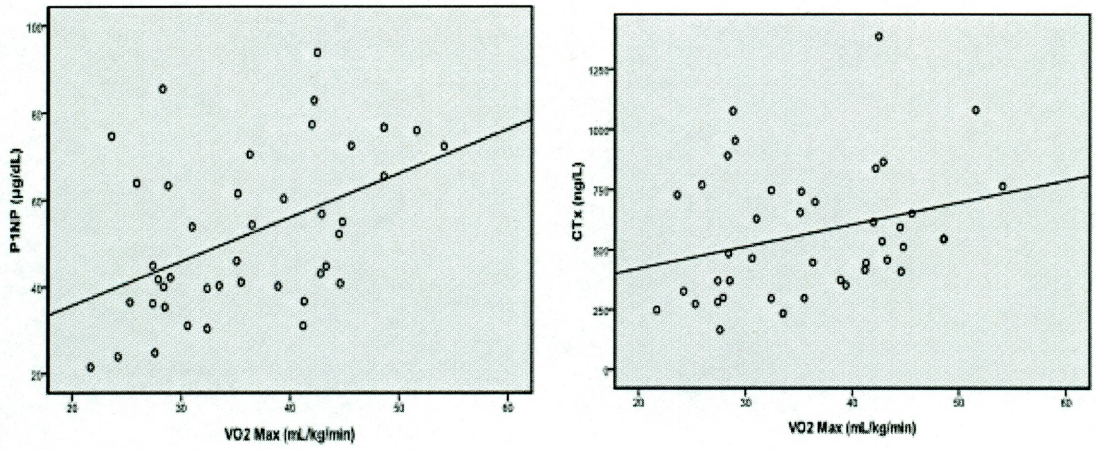

Figure 11. Linear relationship between VO_2 Max and P1NP (on left) and sCTx (on right) (n=42)

Discussion

Results of this study showed that VO_2 Max and sCTx were significant predictors of P1NP. The findings of this study support those of Adami et al, (2008) who reported higher P1NP levels with greater self-reported physical activity and this relationship remained significant after adjustment for age and BMI. However, Brahm et al, (1997) found no significant relationship between VO_2 Max and other markers of bone turnover Procollagen type 1 C-terminal propeptide (P1CP) and the carboxyterminal telopeptide of type 1 collagen (ICTP) in premenopausal women (Brahm et al, 1997). Possible reasons for these differences in the findings could be due to the more recent advancements in assay precision and a larger sample size in this present study. The association of P1NP and fitness suggests that individuals who regularly engage in weight-bearing exercise stimulate bone formation and bone remodeling at higher rate than those who do not exercise. This could be directly due to the mechanical loads placed on bone during weight-bearing exercise or indirectly through other endogenous mechanisms. While the

purpose of this study was not to determine how fitness relates to P1NP, the importance of the findings of this study is the potential use of fitness in predicting biomarkers of collagen remodeling. Further studies with long-term follow-up are needed to determine the potential use for this model in identifying osteoporosis risk.

Conclusion

This study has proposed a unique prediction model for bone formation P1NP which includes VO_2 Max and sCTx. The prediction model demonstrates that fitness has a significant role in evaluating bone remodeling in premenopausal women. Determining fitness in this population could facilitate the identification of risk factors for osteoporosis by providing insight into the rate of bone remodeling.

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CHAPTER FIVE

SUMMARY AND CONCLUSIONS

Osteoporosis affects more than half of the United States population over 50 years of age and 80 percent of those diagnosed with osteoporosis are women. Optimizing bone health through with regular aerobic weight-bearing physical activity is recommended for women prior to menopause so to utilize the osteogenic effect of estrogen on bone. Diagnosed clinically by measuring bone mineral density with Dual Energy X-ray Absorptiometry, this technique is limited as it cannot adequately determine the mechanical properties of bone or measure the rate of collagen remodeling which all contribute to bone strength and subsequent fracture risk. Biomarkers of bone turnover, the formation and degradation of type 1 collagen (P1NP and CTx), have been researched in postmenopausal women for the efficacy of pharmacological interventions, yet few studies have measured these biomarkers in premenopausal women or investigated the response of biomarkers of bone turnover to exercise. The research included in this dissertation is novel and significantly contributes to the body of osteoporosis literature in that it investigates biomarkers of bone turnover in premenopausal women with a wide range of fitness, determined by VO_2 Max.

We have chosen to submit the first research paper to the Osteoporosis International journal as the this study supports the efficacy of weight-bearing aerobic

exercise in promoting bone remodeling with greater responses in individuals who are maintaining higher fitness levels.

We have chosen to submit the second paper to the Medicine and Science in Sports in Exercise journal. This study found significant correlations between biomarkers of bone turnover and VO_2 Max and subsequently a prediction model was developed for the bone formation marker P1NP in this population. Determining appropriate levels of fitness in premenopausal women could facilitate the identification of risk factors of osteoporosis by providing insight into the rate of bone remodeling.

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APPENDIX A

ADDITIONAL TABLES AND FIGURES (PUBLISHABLE PAPER I)

Table 1. Mean (SE) of CBC results before, after and 30mins after exercise in the trained group (n=21)

Blood Count	Pre-exercise	Post-exercise	30mins Post-exercise	*p* Value[+]	*p* Value[*] (pre vs. post)	*p* Value[*] (post vs. 30mins post)	*p* Value[*] (pre vs. 30mins post)
WBC[^]	5.95(0.43)	6.31(1.14)	5.57(1.00)	.15	NS	.02	NS
RBC	4.61(0.07)	3.15(0.50)	3.04(0.48)	.02	.08	.003	.001
HGB	14.0(0.26)	9.58(1.52)	9.25(1.47)	.02	.05	.002	NS
HCT	40.6(0.7)	41.4(0.6)	40.1(0.6)	.04	.08	.004	.002
MCV	88.0(0.97)	58.9(9.33)	59.3(9.37)	.03	.01	.01	.07
MCH	30.3(0.36)	20.3(3.22)	20.3(3.22)	.02	.01	NS	.01
MCHC	34.4(0.09)	23.0(3.63)	22.9(3.63)	.001	.01	NS	.01
RDW	11.9(0.2)	7.8(1.23)	7.8(1.23)	.02	.01	NS	.01
PLT	279(15)	221(36)	182(31)	.001	NS	.001	.003
MPV	7.98(0.10)	5.50(0.88)	5.48(0.87)	NS	NS	NS	NS
PCT	0.221(0.01)	0.182(0.03)	0.147(0.02)	.004	NS	.001	.04
PDW	12.6(0.27)	8.9(1.43)	8.8(1.43)	NS	NS	NS	NS
NEU%	53.8(1.75)	42.9(6.96)	46.5(7.45)	.01	NS	.001	NS
LYM%	35.1(1.61)	17.5(3.16)	14.2(2.47)	<.001	<.001	.001	<.001
MON%	8.11(0.23)	4.84(0.79)	4.81(0.79)	<.001	<.001	NS	<.001
EOS%	2.46(0.23)	0.99(0.19)	0.86(0.17)	<.001	<.001	.01	<.001
BAS%	0.480(0.03)	0.391(0.07)	0.305(0.05)	.01	NS	.01	.004
NEU#	2.38(0.45)	4.15(0.79)	3.96(0.75)	<.001	.001	NS	.001
LYM#	1.36(0.23)	1.56(0.28)	1.11(0.19)	.001	.05	.002	.001
MON#	0.340(0.06)	0.468(0.09)	0.402(0.08)	.01	.003	.02	.06
EOS#	0.093(0.02)	0.094(0.02)	0.070(0.01)	<.001	NS	.001	.001
BAS#	0.022(0.005)	0.040(0.01)	0.026(0.005)	<.001	.002	.003	NS
ALY%	0.529(0.09)	0.562(0.01)	0.491(0.08)	.16	NS	.05	NS
LIC%	0.462(0.09)	0.771(0.15)	0.700(0.14)	.002	.001	.10	.01
ALY#	0.034(0.01)	0.053(0.01)	0.040(0.01)	.001	.003	.01	.07
LIC#	0.032(0.008)	0.081(0.02)	0.085(0.01)	<.001	.001	.002	<.001

[+]*p* Values were calculated using Friedman's ANOVA

[*]*p* Values were calculated using Wilcoxon Signed-Ranked tests

[^] WBC=White blood cells; RBC=Red blood cells; HGB=Hemoglobin; HCT=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration; RDW=Red blood cell distribution width; PLT=Platelets; MPV=Mean platelet volume; PCT=Platelet count; PDW=Platelet distribution width; NEU%=Neutrophil percentage; LYM%=Lymphocyte percentage; MON%=Monocyte percentage; EOS%=Eosinophil percentage; BAS%=Basophil percentage; NEU#=Neutrophils; LYM#=Lymphocytes; MON#=Monocytes; EOS#=Eosinophils; BAS#=Basophils; ALY%=Atypical lymphocyte percentage; LIC%=Large immature cells percentage; ALY#=Atypical lymphocyte; LIC#=Large immature cells

Table 2. Mean (SE) of CBC results before, after and 30mins after exercise in the untrained group (n=14)

Blood Count	Pre-exercise	Post-exercise	30mins Post-exercise	*p* Value[+]	*p* Value[*] (pre vs. post)	*p* Value[*] (post vs. 30mins post)	*p* Value[*] (pre vs. 30mins post)
WBC ^	6.26(0.40)	8.34(0.57)	8.55(0.50)	<.001	.001	NS	.003
RBC	4.68(0.15)	4.80(0.15)	4.69(0.14)	.03	.02	.01	NS
HGB	13.4(0.33)	13.8(0.29)	13.4(0.30)	.03	.02	.02	NS
HCT	39.1(0.9)	40.1(0.8)	39.4(0.7)	.03	.03	.03	NS
MCV	84.2(2.2)	84.2(2.2)	83.6(2.2)	.03	NS	.03	.03
MCH	28.9(0.84)	29.0(0.85)	28.8(0.87)	NS	NS	NS	NS
MCHC	34.2(0.17)	34.3(0.18)	34.1(0.22)	.05	.13	.02	.15
RDW	12.2(0.30)	12.1(0.24)	12.1(0.28)	NS	.11	NS	NS
PLT	288(12)	311(22)	289(11)	.07	.12	.12	NS
MPV	8.19(0.25)	8.46(0.28)	8.28(0.23)	.12	.05	NS	NS
PCT	0.234(0.01)	0.256(0.01)	0.237(0.01)	.14	.11	.08	NS
PDW	13.0(0.70)	14.1(0.91)	13.8(0.60)	NS	.06	NS	NS
NEU%	55.1(1.7)	63.7(1.4)	68.5(1.4)	<.001	.001	.01	.001
LYM%	33.2(1.6)	25.8(1.3)	21.0(1.2)	<.001	.001	.01	.001
MON%	8.51(0.34)	7.91(0.35)	8.21(0.33)	.18	.04	NS	NS
EOS%	2.74(0.36)	1.96(0.33)	1.76(0.30)	<.001	.001	.11	.001
BAS%	0.514(0.05)	0.543(0.04)	0.479(0.03)	NS	NS	.05	NS
NEU#	3.47(0.25)	5.36(0.44)	5.89(0.39)	<.001	.001	.15	.002
LYM#	2.06(0.13)	2.12(0.14)	1.78(0.14)	.01	NS	.01	.01
MON#	0.53(0.03)	0.650(0.04)	0.704(0.05)	.02	.01	NS	.01
EOS#	0.177(0.03)	0.167(0.03)	0.155(0.03)	.003	.17	.11	.01
BAS#	0.032(0.004)	0.046(0.005)	0.042(0.005)	.04	.01	NS	.10
ALY%	0.836(0.06)	0.900(0.06)	0.757(0.05)	.08	NS	.04	NS
LIC%	0.793(0.05)	1.15(0.10)	1.16(0.10)	.002	.004	NS	.01
ALY#	0.034(0.006)	0.072(0.005)	0.064(0.006)	.01	.003	.18	.02
LIC#	0.032(0.008)	0.102(0.02)	0.103(0.01)	.002	.001	.001	.01

[+] *p* Values were calculated using Friedman's ANOVA
[*] *p* Values were calculated using Wilcoxon Signed-Ranked tests
^ WBC=White blood cells; RBC=Red blood cells; HGB=Hemoglobin; HCT=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration; RDW=Red blood cell distribution width; PLT=Platelets; MPV=Mean platelet volume; PCT=Platelet count; PDW=Platelet distribution width; NEU%=Neutrophil percentage; LYM%=Lymphocyte percentage; MON%=Monocyte percentage; EOS%=Eosinophil percentage; BAS%=Basophil percentage; NEU#=Neutrophils; LYM#=Lymphocytes; MON#=Monocytes; EOS#=Eosinophils; BAS#=Basophils; ALY%=Atypical lymphocyte percentage; LIC%=Large immature cells percentage; ALY#=Atypical lymphocyte; LIC#=Large immature cells

Table 3. Mean (SE) of CBC results of the trained and untrained groups before, after and 30mins after exercise (n=35)

Blood Count	**Pre-exercise** Trained (n_1=21)	**Pre-exercise** Untrained (n_2=14)	***p* Value**[+]	**Post-exercise** Trained (n_1=21)	**Post-exercise** Untrained (n_2=14)	***p* Value**[+]	**30mins Post-exercise** Trained	**30mins Post-exercise** Untrained	***p* Value**[+]
WBC[^]	5.95(0.43)	6.26(0.40)	NS	6.31(1.14)	8.34(0.57)	NS	5.57(1.00)	8.55(0.50)	.06
RBC	4.61(0.07)	4.68(0.15)	NS	3.15(0.50)	4.80(0.15)	.06	3.04(0.48)	4.69(0.14)	**.05**
HGB	14.0(0.26)	13.4(0.33)	.20	9.58(1.52)	13.8(0.29)	NS	9.25(1.47)	13.4(0.30)	NS
HCT	40.6(0.7)	39.1(0.9)	.19	41.4(0.6)	40.1(0.8)	NS	40.1(0.8)	39.4(0.7)	NS
MCV	88.0(0.97)	84.2(2.2)	.14	58.9(9.33)	84.2(2.2)	NS	59.3(9.37)	83.6(2.2)	.06
MCH	30.3(0.36)	28.9(0.84)	.10	20.3(3.22)	29.0(0.85)	NS	20.3(3.22)	28.8(0.87)	NS
MCHC	34.4(0.09)	34.2(0.17)	NS	23.0(3.63)	34.3(0.18)	.15	22.9(3.63)	34.1(0.22)	NS
RDW	11.9(0.2)	12.2(0.30)	NS	7.8(1.23)	12.1(0.24)	**.01**	7.8(1.23)	12.1(0.28)	**.01**
PLT	279(15)	288(12)	NS	221(36)	311(22)	.19	182(31)	289(11)	**.05**
MPV	7.98(0.10)	8.19(0.25)	.18	5.50(0.88)	8.46(0.28)	**.04**	5.48(0.87)	8.28(0.23)	.07
PCT	0.221(0.01)	0.234(0.01)	NS	0.182(0.03)	0.256(0.01)	.14	0.147(0.02)	0.237(0.01)	**.03**
PDW	12.6(0.27)	13.0(0.70)	NS	8.9(1.43)	14.1(0.91)	**.04**	8.8(1.43)	13.8(0.60)	**.02**
NEU%	53.8(1.75)	55.1(1.7)	NS	42.9(6.96)	63.7(1.4)	.18	46.5(7.45)	68.5(1.4)	NS
LYM%	35.1(1.61)	33.2(1.6)	NS	17.5(3.16)	25.8(1.3)	.11	14.2(2.47)	21.0(1.2)	.09
MON%	8.11(0.23)	8.51(0.34)	NS	4.84(0.79)	7.91(0.35)	**.01**	4.81(0.79)	8.21(0.33)	**.001**

Table 3. Continued

EOS%	2.46(0.23)	2.74(0.36)	NS	0.99(0.19)	1.96(0.33)	**.01**	0.86(0.17)	1.76(0.30)	**.01**
BAS%	0.480(0.03)	0.514(0.05)	NS	0.391(0.07)	0.543(0.04)	NS	0.305(0.05)	0.479(0.03)	**.05**
NEU#	2.38(0.45)	3.47(0.25)	**.05**	4.15(0.79)	5.36(0.44)	NS	3.96(0.75)	5.89(0.39)	.10
LYM#	1.36(0.23)	2.06(0.13)	.08	1.56(0.28)	2.12(0.14)	NS	1.11(0.19)	1.78(0.14)	**.04**
MON#	0.340(0.06)	0.53(0.03)	**.03**	0.468(0.09)	0.650(0.04)	.13	0.402(0.08)	0.704(0.05)	**.002**
EOS#	0.093(0.02)	0.177(0.03)	**.02**	0.094(0.02)	0.167(0.03)	**.05**	0.070(0.01)	0.155(0.03)	**.003**
BAS#	0.022(0.005)	0.032(0.004)	.15	0.040(0.01)	0.046(0.005)	NS	0.026(0.005)	0.042(0.005)	**.03**
ALY%	0.529(0.09)	0.836(0.06)	**.04**	0.562(0.01)	0.900(0.06)	**.03**	0.491(0.08)	0.757(0.05)	.06
LIC%	0.462(0.09)	0.793(0.05)	**.01**	0.771(0.15)	1.15(0.10)	.08	0.700(0.14)	1.16(0.10)	**.03**
ALY#	0.034(0.01)	0.034(0.006)	.07	0.053(0.01)	0.072(0.005)	.19	0.040(0.01)	0.064(0.006)	NS
LIC#	0.032(0.008)	0.032(0.008)	**.02**	0.081(0.02)	0.102(0.02)	.16	0.085(0.01)	0.103(0.01)	NS

[1] *p* Values were calculated using Mann-Whitney U-tests

[2] WBC=White blood cells; RBC=Red blood cells; HGB=Hemoglobin; HCT=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration; RDW=Red blood cell distribution width; PLT=Platelets; MPV=Mean platelet volume; PCT=Platelet count; PDW=Platelet distribution width; NEU%=Neutrophil percentage; LYM%=Lymphocyte percentage; MON%=Monocyte percentage; EOS%=Eosinophil percentage; BAS%=Basophil percentage; NEU#=Neutrophils; LYM#=Lymphocytes; MON#=Monocytes; EOS#=Eosinophils; BAS#=Basophils; ALY%=Atypical lymphocyte percentage; LIC%=Large immature cells percentage; ALY#=Atypical lymphocyte; LIC#=Large immature cells