THE EFFECT OF ACUTE RESISTANCE EXERCISE VOLUME THRESHOLD ON BIOMARKERS OF BONE METABOLISM

A THESIS

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Kristin Helton entitled "The Effects of Acute Resistance Exercise Volume Threshold on Biomarkers of Bone Metabolism." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Exercise and Sports Nutrition.

David L. Nichols, Major Professor

We have read this thesis and recommend its acceptance:

Department Chair

Accepted:

Dean of the Graduate School

DEDICATION

To my parents, Larry and Karen Helton,
Thank you for your support, love and encouragement.

To my husband, Dr. David Pham,
Thank you for your love and inspiration.

To my brothers, Major Michael Helton and Eric Helton,
Thank you for your love and guidance.

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ABSTRACT

KRISTIN HELTON

THE EFFECT OF ACUTE RESISTANCE EXERCISE VOLUME THRESHOLD ON BIOMARKERS OF BONE METABOLISM

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The purpose of this study was to determine the effects of two different volumes of resistance exercise on biomarkers of bone metabolism.

Ten premenopausal women (18-40 yrs) performed two bouts of resistance exercise, one low volume (1 set of 10 exercises) and one high volume (3 sets of 10 exercises) bout. Serum was collected 24 hr after each bout and rest to measure biomarkers of bone formation (osteocalcin and bone-specific alkaline phosphatase [BAP]) and bone resorption (C-terminal collagen cross-links [CTX] and tartrate-resistant acid phosphatase [TRAP5b]). Changes in each marker and in the ratio of bone formation to bone resorption were compared between each treatment and rest.

There was no significant difference between rest and the two bouts of exercise in any markers or in the ratio of bone formation to bone resorption. In conclusion, a bout of resistance exercise has no effect on biomarkers of bone metabolism after 24 hours.

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CHAPTER I

INTRODUCTION

In his novel, *Great Expectations*, Charles Dickens noted of the elderly Miss Havisham that "Her chest had drooped, so that she stooped." The stature of a stooped older woman has been described throughout history through paintings, words in a novel, and has even been found in 4000 year old Egyptian mummies (Patlak, 2001). This phenomenon known as a "dowager's hump" has, until recently, been thought to be a natural process of aging. It is now known to be a result of osteoporosis, a skeletal disease characterized by low bone mass and structural deterioration of bone tissue, resulting in an increased risk of fracture. The fractures that cause the formation of a "dowager's hump" may be brought about by as little as a sneeze or a cough (Weber, Uehlinger, & Gerber, 2002). While the term "osteoporosis" had not yet been defined, the disease has been investigated since the early 1770s, when John Hunter discovered that old bone was destroyed as new bone was formed (Patlak).

Over the 200 years since this discovery, osteoporosis has become a major health threat for 44 million Americans with 68% of those being women (U.S. Department of Health & Human Services, 2004). While 10 million of these Americans already suffer from osteoporosis, 34 million individuals have low bone mass, or osteopenia, increasing their risk for osteoporosis. In other words, one in two women and one in four men will have an osteoporosis-related fracture in their lifetime. In 2004, the Surgeon General reported 1.5

million fractures from osteoporosis, 500,000 hospitalizations, 800,000 trips to the emergency room, 2.6 million doctor visits, and 180,000 Americans placed in a nursing home due to osteoporosis, costing America $18 billion each year (U.S. Department of Health & Human Services). Osteoporosis is most common among postmenopausal women and elderly men. As life expectancy continues to increase, so will the number of those who suffer from osteoporosis. Therefore, current research lies in disease prevention.

While bone mass continues to increase in earlier years, it reaches its peak around the age of 30 years for most individuals after which point bone mineral density (BMD) begins to decrease. It is, therefore, important for individuals to achieve maximal peak bone mass during earlier years of life to prevent osteoporosis. Factors that affect bone mass include gender, age, hormone levels, nutrition, and exercise. Although the former three cannot be altered by lifestyle changes, diet and exercise have a dramatic effect on achieving peak bone mass and maintaining BMD later in life (WHO, 2003). While previous research has focused on postmenopausal women when bone loss has already been accelerated due to hormone changes, premenopausal women have become the focus of current research.

No matter what age an individual may be, physical activity, especially weight bearing activity, has improved bone health and decreased bone loss in humans. High-impact aerobic exercise training improves bone metabolism in healthy sedentary individuals, trained individuals, and in those at risk of an osteoporotic fracture. Direct measurement of bone density (by dual energy x-ray absorptiometry) has revealed site specific effects of

endurance running on the hip. This was a result of a greater magnitude of loading on the hip due to running that prevented the loss of bone mass in the hip, but not the spine (Hind, Truscott, & Evans, 2006). Acute bouts of aerobic exercise act transiently in favor of bone formation by decreasing bone resorption (Woitge et al., 1998). In contrast, the benefits of resistance training are thought to be achieved through an increase in bone formation (Fujimura et al., 1997; Woitge et al.). Resistance exercise may also improve muscle strength as well as the quality of life in elderly individuals who may not be able to tolerate aerobic exercise.

While both aerobic and resistance exercises provide beneficial effects to bone metabolism, a combination of the two types of exercise is most effective in achieving optimal bone health (Hind et al., 2006). Resistance exercise may improve bone turnover with higher intensities (Kristofferson, Hultdin, Holmlund, Thorsen, & Lorentzon, 1995; Vincent & Braith, 2002; Whipple et al., 2004). However, no studies to date have investigated the effects of different volumes of exercise on biomarkers of bone metabolism. Future research goals should include identifying the type of exercise, intensity, and volume that results in improved bone health.

Purpose of the Study

The purpose of this study was to compare the response of markers of bone formation (osteocalcin and bone-specific alkaline phosphatase) and markers of bone resorption (C-terminal collagen cross-links and tartrate-resistant acid phosphatase) at rest to a single-bout of resistance exercise performing 1 set of 10 exercises (RE1) and a single-bout of resistance exercise performing 3 sets of 10 exercises (RE3) in sedentary women. The

participants consisted of 10 premenopausal women (age 18-40). The study implemented a repeated measures design. Each treatment (rest, RE1, RE3) was separated by 25-32 days to control for variations in the menstrual cycle, and serum was collected from the participants 24 hr following the intervention for a total of three blood draws. A conclusion was drawn about the acute effects of resistance exercise volume on markers of bone turnover.

Hypothesis

The following null hypotheses were examined at a 0.025 level of significance.

1. There was no difference in markers of bone turnover between RE1, RE3, and rest in 10 premenopausal women.
2. There was no difference in the ratio of bone formation to bone resorption markers in RE1, RE3, and rest in 10 premenopausal women.

Definitions

The following definitions are presented to guide the reader.

1. Aerobic exercise: any activity that uses large muscle groups, can be maintained continuously, and is rhythmic and aerobic in nature (ACSM, 2006).

2. Alkaline phosphatase: an enzyme associated with the plasma membrane of osteoblasts, but is also found in the liver, intestine and placenta (Moss, 1987). Bone alkaline phosphatase makes up about 45% of serum concentrations of alkaline phosphatase (Watts, 1999).

3. Areal bone mineral density (aBMD): a measurement of bone density that integrates the size, thickness, and true volumetric density of the bone. It is found by

dividing the bone mineral content by the area of the image of bone projected in two dimensions (Cummings, Bates, & Black, 2002).

4. Bone formation: the process by which osteoblasts deposit new bone within the existing bone matrix. It consists of three phases: the production and the maturation of osteoid matrix followed by the mineralization of the matrix (Hadjikadis & Androulakis, 2006).

5. Bone mineral content (BMC): the amount of mineral measured in a defined section of the bone. The mineral content of the whole skeleton is referred to as total body bone mineral content (Cummings et al., 2002).

6. Bone resorption: the process during which osteoclasts erode old bone from the existing bone matrix so that new bone can be formed (Hadjidakis & Androulakis, 2006).

7. Cortical bone: hard, compact bone found mainly in the shafts of long bones that makes up 80% of the skeleton (Hadjidakis & Androulakis, 2006).

8. Cross-linked telopeptides: amino- (NTx) and carboxy- (CTx) terminal fragments of collagen that are released during bone resorption with collagen cross-links attached. CTx is also referred to in some papers as carboxy-terminal telopeptide of type-1 collagen or ICTP (Watts, 1999).

9. Dual-energy x-ray absorptiometry (DXA): the "gold standard" in diagnosing osteoporosis and reports the areal bone mineral density. It works by measuring the transmission through the body of x-rays of two different energy levels (WHO, 2003).

10. Osteoblast: bone-forming cells that originate from mesenchymal stem cells (Hadjidakis & Androulakis, 2006).

11. Osteocalcin: the major noncollagen protein of the bone matrix; used as a marker of bone formation (WHO, 2003).

12. Osteoclast: bone-resorbing cells located at the surface of a calcified bone and the lacuna (Teitelbaum, 2000).

13. Osteopenia: reduced bone mineral density defined by a BMD value 1 to 2.5 standard deviations below the young adult mean (WHO, 2003).

14. Osteoporosis: a condition of reduced bone mineral density associated with an increased risk of fracture and defined by a BMD value 2.5 standard deviations below the young adult mean (WHO, 2003).

15. Parathyroid hormone: a hormone released from the parathyroid glands in response to low concentrations of calcium and phosphate (Hadjidakis & Androlakis, 2006).

16. Peak bone mass: the highest amount of bone mass that a person achieves during their lifetime. This usually occurs around the age of 30 (WHO, 2003).

17. Procollagen extension peptides: the amino (PINP) and carboxy (PICP) terminal products of procollagen molecules secreted from osteoblasts. They are used as markers of bone formation (Scariano et al., 1998).

18. Pyridinium cross-links: collagen breakdown products that include the cross-links pyridinoline (Pyd) and deoxypyridinoline (Dpd). These cross-links are released with the resorption of type 1 collagen and are markers of bone resorption (Eyre, 1987).

19. Tartrate-resistant acid phosphatase (TRAP): a lysosomal enzyme found in bone that is used as a marker of bone resorption. The nonsialyliated isoform, TRAP5b, is

a cleaved two-subunit form derived from osteoclasts and is the form used in this study (Watts, 1999).

20. Trabecular bone: also referred to as cancellous or spongy bone and is found mainly at the ends of long bones and within the vertebrae (Hadjidakis & Androulakis, 2006).

21. T-score: a score used in the diagnosis of osteoporosis and is expressed in standard deviations. It is determined by comparing the measured BMD to the young adult mean (WHO, 2003).

22. Z-score: a less commonly used score to define osteoporosis based on the age-matched mean (WHO).

Assumptions

1. The participants in the study tried their best during the exercise sessions.
2. The sample of women is representative of all premenopausal women in the United States.
3. The analysis of the serum was accurate.
4. The assessment tools and measurement process used was valid and reliable.

Limitations

1. The external validity of the study can only be applied to sedentary, disease-free, female population between the ages of 18 and 45.
2. Only 10 participants completed the study.

Significance

At present, doctors depend on the use of dual-energy x-ray absorptiometry to diagnose osteoporosis as well as measure the effectiveness of an intervention. While changes in bone density as assessed by DXA can sometimes take over 6 months to become detectable, biochemical markers of bone metabolism can be used to detect both acute and long-term effects of an intervention. This medical advancement may prove to be an important tool in measuring the progress of a bone density treatment as well as determining the best methods of preventing low bone mass. Resistance exercise has a positive effect on BMD at the loaded skeletal sites (Fujimura et al., 1997; Heinonen, Sievanen, Kannus, Oja, & Vuori, 2002; Shackelford et al., 2004; Vincent & Braith, 2002; Winter-Stone & Snow, 2006). There is a positive impact of resistance exercise on markers of bone turnover (Fujimura et al.; Shackelford et al.; Tosun, Bolukbast, Cingi, Beyazova, & Unlu, 2006; Vincent & Braith, 2002; Whipple et al., 2004). Although it has been established that resistance exercise helps improve bone density, the question lies in how much is needed to cause the greatest increases in BMD. However, the effects of any volume of resistance exercise on BMD will take several months to be detected whereas the response of markers of bone metabolism to exercise might be seen in only a few days. Therefore, the purpose of this study was to compare the acute effects of two different exercise volumes (1 set vs. 3 sets) to resting values of markers of bone turnover.

CHAPTER II

REVIEW OF THE LITERATURE

The purpose of the study is to compare the effect of two different resistance exercise volumes on biological markers of bone formation and resorption. This chapter will review the research literature pertaining to the following topics: (a) the physiology of normal bone metabolism, (b) diagnosis and assessment of osteoporosis and bone metabolism, (c) nutritional influences on bone health, (d) the effects of aerobic exercise on bone metabolism, (e) the effects of resistance training on bone metabolism, and (f) nutrition and exercise interactions.

The Physiology of Normal Bone Metabolism

The mature human skeleton is comprised of two types of bone: cortical and trabecular. Cortical bone is compact bone that makes up 80% of the skeletal mass and constitutes the outer part of all skeletal structures. It has a slow turnover rate and is highly resistant to bending and torsion (Hadjidakis & Androulakis, 2006). This cortical sheath functions to provide mechanical strength and protection to the trabecular network that it encloses. Trabecular bone represents only 20% of the skeletal mass and has a higher turnover rate than cortical bone. The trabecular network is made up of interconnected rods and plates that are preferentially oriented along the lines of mechanical strain to maximize strength and minimize weight (World Health Organization [WHO], 2003). The ratio of cortical to trabecular bone varies depending on the skeletal location. In the lumbar spine, femoral

neck, and radial diaphysis, trabecular bone accounts for 70, 50, and 5% of the total bone tissue, respectively (Einhorn, 1996).

The bone matrix consists mainly of type 1 collagen fibers and noncollagenous proteins. Crystals of hydroxyapatite are located on these collagen fibers as well as within them and in the matrix. This mineral is also oriented in the same direction as the collagen fibers. Of the noncollagenous proteins produced, osteocalcin (GLA protein) is the most predominant. Other noncollagenous proteins include osteonectin, osteopontin, and bone sailoprotein (WHO, 2003). Osteocalcin has been shown to play a role in calcium binding, hydroxyapatite stabilization in the matrix, and negative regulation of bone formation (Hadjidakis & Androulakis, 2006). Its role as a negative regulator of bone formation serves to inhibit premature or inappropriate mineralization. These noncollagenous matrix proteins are synthesized and laid down by osteoblasts.

Osteoblasts are one of three types of bone cells that participate in bone remodeling. Osteoblasts are bone-forming cells that originate form mesenchymal stem cells (WHO, 2003). These stem cells have the potential to differentiate into osteoblasts, adipocytes, chondrocytes, myoblasts, or fibroblasts (Ducy, Debois, & Boyce, 1996). Osteoblasts function in clusters along the bone surface and line the layer of bone matrix that they are producing. Bone formation occurs in three phases: the production and the maturation of osteoid matrix followed by the mineralization of the matrix (Hadjidakis & Androulakis, 2006). Fifteen percent of the mature osteoblasts used in bone formation are entrapped in the new bone matrix and then differentiate into osteocytes. Some osteoblasts also remain on the bone surface and become flat lining cells (Hadjidakis & Androulakis). In normal

adult bone, the three phases occur at the same rate so that a balance exists between matrix production and mineralization (Hadjidakis & Androulakis). Bone mineral ions such as calcium and phosphate are transported from the extracellular space of the bone marrow to the osteoid layer via transport systems in the plasma membrane of osteoblasts (Hadjidakis & Androulakis). This plasma membrane is also rich in alkaline phosphatase, which enters the systemic circulation, making it a useful biochemical marker of bone formation (WHO, 2003). Another function of osteoblasts includes the production of different growth factors, including insulin-like growth factors (IGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β), and bone morphogenic proteins (BMP) (Hadjidakis & Androulakis). Osteoblastic activity is thought to be regulated by these hormones as well as other hormones that have receptors on osteoblasts such as parathyroid hormone (PTH), parathyroid hormone-related protein, thyroid hormone, growth hormone, insulin, progesterone, and prolactin. Osteoblasts also have steroid hormone receptors for estrogens, androgens, vitamin D_3, and retinoids (Hadjidakis & Androulakis).

Of the growth factors produced from osteoblasts, insulin-like growth factor I (IGF-I) and thyroid hormones have sparked interest in their role as regulators of skeletal development. IGF-I is synthesized mainly in the liver with its synthesis being highly dependent on growth hormone (GH). However, it is also produced by osteoblasts in the cortical bone and is positively correlated with BMD (Gillberg et al., 2002). Studies have shown that IGF-I induces bone remodeling through the expression of osteoclasts and osteoblasts, with it primarily having an osteogenic effect (Tokimasa et al., 2003). Thyroid

hormones (T3/T4) have been shown to enhance bone growth in children, but can result in bone loss in adults. Hyperthyroidism results in increased bone turnover, with markers of bone resorption exceeding those of bone formation, and resulting in a loss of cortical and trabecular bone (Garnero, Vassy, Bertholin, Riou, & Delmas, 1994; Huang, Golden, Tarjan, Madison, & Stern, 2000). Several studies have demonstrated the ability of T_3 to increase osteoblastic markers and that IGF-I was an essential factor for the anabolic effects of T_3 on the bone (Huang et al.).

After osteocytes are differentiated from osteoblasts, they function to form a network of thin canaliculi that permeates the entire matrix. Functional activity of osteocytes decreases with age, with the older osteocytes being located deep within the calcified bone. Older osteocytes also present a decreased cell volume and an increased glycogen accumulation in the cytoplasm (Hadjidakis & Androulakis, 2006). Osteocytes are thought to play a role in the homeostasis of extracellular fluid as well as its role in local activation of bone remodeling in response to mechanical loads (WHO, 2003).

The third cell type is osteoclasts, also known as bone-resorbing cells. These cells contain 4-20 nuclei and can be up to 100 mm in diameter. They are derived from hematopoietic cells of the mononuclear lineage and are usually located at the surface of a calcified bone and the lacuna (Teitelbaum, 2000). The function of osteoclasts is mainly due to their formation of the deep folding of the plasma membrane in the area facing the bone matrix known as the ruffled border. This ruffled border secretes osteoclast synthesized enzymes such as tartrate-resistant acid phosphatase and cathepsin K. Osteoclasts perform the bone-resorbing role by "acidification and proteolysis of the bone

matrix and of the hydroxyapatite crystals encapsulated within the sealing zone" (Hadjidakis & Androulakis, 2006, p. 389). The resorption process consists of the mobilization of the hydroxyapatite crystals by digestion of their link to collagen followed by the digestion of residual collagen fibers by cathepsins or activated collagenases (Hadjidakis & Androulakis). The residues from this digestion are then released at the basolateral domain. Osteoclast cell activity is regulated by locally acting cytokines as well as systemic hormones. Osteoclast receptors have been identified for calcitonin, androgens, thyroid hormone, insulin, PTH, IGF-1, interleukin-1 (IL-1), colony stimulating factor-1 (CSF-1), and platelet-derived growth factor (PDGF). Parathyroid hormone is a hormone of interest in the development of osteoporosis. This hormone is released from the parathyroid glands in response to low concentrations of calcium and phosphate. Parathyroid hormone then stimulates the mobilization of calcium from the bone through the action of osteoclasts. At the same time, PTH acts on the kidney to increase calcium reabsorption as well as increase the production of the active form of vitamin D in order to increase calcium absorption in the small intestine. Therefore, concentrations of PTH may serve as useful marker of bone metabolism.

Bone Remodeling and Osteoporosis

Bone remodeling is a process that occurs throughout adult life that replaces old bone with new bone. Each year, remodeling is responsible for replacing 25% of trabecular bone and about 3% of cortical bone (Watts, 1999). This process consists of three consecutive phases that include resorption, reversal, and formation. Resorption can last from a few days (WHO, 2003) to a couple of weeks ((Hadjidakis & Androulakis, 2006)).

The reversal phase may last 4 to 5 weeks, and bone formation may last up to 4 months. During this remodeling process, osteoclasts and osteoblasts form a basic multicellular unit (BMU). In the cortical bone, the BMU forms a cylindrical canal that burrows through the bone at a speed of 20-40 μm/day while trabecular bone is remodeled at a speed of 25 μm/day. During one cycle, only 10 osteoclasts are responsible for digging a circular tunnel in the dominant loading direction while it takes thousands of osteoblasts to fill the tunnel. After remodeling is complete, there is a resting phase before the next cycle begins. Part of the process of remodeling is to enable calcium phosphate to be excreted from the skeleton whenever the net intestinal absorption of calcium is less than that excreted in the urine (WHO, 2003). However, whenever the amount of bone resorbed is not in balance with the amount formed, bone loss occurs leaving the skeleton less mechanically sound. After peak bone mass is reached between the ages of 18 and 30, bone loss begins, and the rate of bone loss increases substantially around the onset of menopause. The greater the amount of bone lost from peak bone mass, the greater a person's risk is for suffering from an osteoporotic fracture. This risk of an osteoporotic fracture is assessed in terms of BMD measured by DXA. Biological markers of bone density are able to give researchers and clinicians a better idea about the changes in bone mineralization that are occurring over a shorter period of time.

Diagnosis and Assessment of Osteoporosis and Bone Metabolism

Osteoporosis is a term derived from the words "osteon", meaning bone, and "poros," referring to a small passage or pore. As defined in the American Journal of Medicine by the Consensus Development Conference (1993), osteoporosis is "a systemic skeletal

disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (American Journal of Medicine, 1993, p. 646). This definition essentially identifies the disease by a risk factor. However, this is necessary due to the majority of fractures only occurring late in the disease when the skeletal integrity is already severely compromised. Therefore, goals for osteoporotic fracture prevention lie at identifying those with high risk in order to begin treatment early (Fogelman & Blake, 2000). The WHO recommended the assessment of these risks and the identification of osteoporosis be based on T-scores of the BMD of the spine, hip, and forearm (WHO, 1994). The T-score is found by comparing the measured BMD to the young adult mean. T-scores were then developed based on the BMD of the hip as assessed by DXA using the NHANES III data. Z-scores, although less commonly used, define osteoporosis based on an age-matched mean (Fogelman & Blake).

Dual-energy X-ray absorptiometry is the "gold standard" for the diagnosis of osteoporosis based on BMD. In addition, only specific skeletal sites should be used for the diagnosis. The BMD at both posterior-anterior (PA) spine and the hip should be measured in all patients. In the case that the hip and/or spine cannot be measured (such as during hyperparathyroidism or in very obese patients), the forearm BMD should be measured (Lewiecki et al., 2004). The PA L1-L4 should be used for the spine, and vertebrae that are affected by local structural change should be excluded. The total proximal femur, femoral neck, or trochanter should be used to assess the hip BMD,

whichever is lowest. Finally, the 33% radius of the nondominant forearm should be used to assess forearm BMD (Lewiecki et al.).

The following table identifies the diagnostic criteria for osteoporosis based on T-scores in certain populations. Because of the high prevalence of osteoporosis among white postmenopausal women, diagnostic criteria was set to capture about 30% of this population (Kanis, 1997). However, the WHO definitions for osteoporosis cannot automatically be applied to other BMD measurement sites, to other technologies, or to other populations (Fogelman and Blake, 2000).

Table 1

Diagnostic Criteria

Population	Diagnostic Criteria for Osteoporosis
Postmenopausal women	T-score of –2.5 or less (based on female reference values)
Premenopausal women (age 20 to menopause)	Z-score of -2.0 or less with secondary causes of BMD
Men aged ≥ 65 years	T-score of –2.5 or less (based on male reference values)
Men aged 50-65 years	T-score of –2.5 or less along with other risk factors for fractures
Men of any age	May be diagnosed if secondary causes of low BMD are present (use Z-scores if <50)

Note. The information in the table was adapted from Leslie et al. (2006), Lewiecki et al. (2004), and Watts (1999).

In the above populations, osteoporosis may be diagnosed if low BMD is accompanied by secondary causes or other risk factors for fractures (i.e. glucocorticoid therapy,

hyperparathyroidism, hypogonadism). Risk factors for osteoporosis also include a history of fracture after the age of 40; a history of a fragility fracture in a first-degree relative; a history of hip, wrist, or vertebral fracture in a first-degree relative; being in the lowest quartile for body weight ($\leq$ 57.8 kg or 127 lb); a current cigarette smoking habit; and the use of oral glucocorticoid therapy for more than three months (Lewiecki et al., 2004; Watts, 1999). Additional osteoporosis risk factors include "impaired vision, estrogen deficiency at an early age (< 45 yr), poor health/frailty, recent falls, low calcium intake (lifelong), low physical activity, and alcohol in amounts more than two drinks per day" (Lewiecki et al., p. 3652). Medications that result in reduced bone mass in adults include anticonvulsants, GnRH agonists, excessive T_4 doses, and lithium (Lewiecki et al.). Unfortunately, the WHO-defined T-score range of osteoporosis falls short of capturing most fractures. In a study by Miller et al. (2002), only 6.4% of 2259 women reporting new fractures had a T-score indicative of osteoporosis. Because all men and women with fractures do not have an osteoporotic T-score, the World Health Organization has identified T-score ranges for individuals at an increased risk for fracture. Those with a T-score between –2.5 and –1 are identified as osteopenic while individuals with T-scores of greater or equal to –1 are classified as healthy. However, the WHO definition of osteopenia "is not useful in isolation with regard to decisions about treatment, because it captures too high a percentage of postmenopausal women (Fogelman and Blake, 2000, p. 2023)." According to the BMD categories, 15% of young healthy women meet the criteria for osteopenia while 0.6% will meet the criteria for osteoporosis (WHO, 2003). If a Z-score is used, a score of less than -2 means that the person is "below the expected

range for age (Leslie et al., 2006, p. 24)." In addition, the WHO study group (1994) proposed another category by which "severe osteoporosis" or "established osteoporosis" is defined by a BMD 2.5 SDs or more below the young adult mean in the presence of one or more fragility fractures. It is important to note that the criterion for osteoporosis is aimed at postmenopausal women and men over 50 years of age. Premenopausal women and younger men should not be diagnosed based on densitometry alone (Singer, 2006). Therefore, premenopausal women and men under the age of 50 should be diagnosed by a Z-score below the expected range along with secondary causes of low BMD (Leslie et al.).

The goal of clinical densitometry is not only to predict the risk of fracture, but to monitor the effectiveness of treatments once the risk has been assessed. To correctly evaluate the risk of incurring a hip fracture, the BMD of the hip would provide the most reliable measurement (Cummings, Black, & Nevitt, 1993; Marshall, Johnell, & Wedel, 1996). In contrast, the spine is optimal for measuring the response to treatment because of the metabolically active trabecular bone that lies in the vertebral bodices (Eastell, 1998).

SXA and DXA

Single and dual-energy x-ray absorptiometry (SXA, DXA) are methods used to assess the bone mineral content of the whole skeleton as well as specific skeletal sites although DXA is known as the "gold-standard" for the non-invasive diagnosis of osteoporosis. "Bone mineral content" is a term that describes the amount of mineral present in a specific bone site. Dividing the bone mineral content by the area or volume of the site measured then derives the "bone mineral density". Single-energy x-ray absorptiometry

and DXA both provide two-dimensional scans which result in the BMD being reported as an areal density (WHO, 2003). In SXA, BMD is only measured at the appendicular sites such as the heel or wrist as well as at the forearm. Single-energy x-ray absorptiometry is more precise than single dual photon absorptiometry (SPA), but neither can be used for the spine or hip (WHO, 2003).

Dual-energy x-ray absorptiometry was developed in the mid-1980s from dual photon absorptiometry (DPA) by replacing the ^{153}Gd radionuclide source with an x-ray tube (Fogelman & Blake, 2000). It works by measuring the transmission through the body of x-rays of two different photon energy levels. Transmisson factors must be measured at two different energy levels to enable the areal densities to be measured of two different tissue types (hydroxyapatite and soft tissue) due the dependency of the attenuation coefficient on the atomic number and photon energy (Fogelman & Blake).

Measurement of BMD by DXA scans results in some error (Binkley et al., 2006). For an individual DXA technologist, the minimum acceptable precision is 1.9% at the lumbar spine, 1.8% at the total hip, and 2.5% at the femoral neck (Binkley et al.). There are many advantages to DXA as opposed to other methods of BMD measurement. These include exposure to very low levels of radiation (1-10 μSv compared to the average daily dose from natural background radiation of 7 μSv), high precision, short scan times, stable calibration, and spine and hip measurement accuracy. The hip is the most important place of measurement due to it being the leading cause of disability in osteoporotic patients. It's also the most reliable in evaluating the risk of hip fracture (Cummings et al., 1993; Marshall et al., 1996). The sensitivity of DXA at measuring the lumbar spine is

significantly greater than the femoral neck. However, this difference is insignificant in men over 60 and women over 70 years of age (Moayyeri, Soltani, & Bahrami, 2006). Therefore, the lumbar DXA is preferred in men < 60 and women < 70 while the hip or femoral DXA may be used in older patients (Lane, 2006; Leweicki et al., 2004).

QUS (Quantitative Ultrasound)

Broad-band ultrasound attenuation (BUA) and speed of sound (SOS) at the heel are the two parameters evaluated by QUS. While they may be useful in evaluating calcaneal systems, they are not useful in diagnosing osteoporosis. The advantages of QUS are that they do not involve ionizing radiation and may be useful in providing information on the structural organization of the bone as well as bone mass (WHO, 2003).

QCT

Quantitative computed tomography (QCT) is a way of assessing bone density that can be applied to the appendicular skeleton and spine (WHO, 2003). Unlike DXA, QCT has the advantage of taking a three-dimensional density as opposed to the two-dimensional taken by DXA. This three-dimensional image allows for a true volumetric density instead of an areal density (Fogelman & Blake, 2000) and results in giving information on the shape and macroarchitecture of the bone. However, the resolution of the cancellous bone isn't optimal (WHO, 2003). In QCT, the patient is scanned in a clinical CT scanner with a calibration phantom for standardization. The volumetric results are then suitable for monitoring treatments due to cancellous bone being more responsive to interventions than cortical bone. Disadvantages of QCT include high radiation exposure, high cost, and difficult quality control when compared to DXA (WHO, 2003).

Other Methods

If DXA or QCT cannot be used, radiography may be sufficient in diagnosing osteoporosis by taking the ratio of the cortical width to the total width to use as an index (WHO, 2003). "Since the size of tubular bones increases with age, thinning of the cortex represents an increase in net endocortical bone resorption" (WHO, 2003, p.56). The distal forearm, distal phalanges, and metacarpals are common sites for radiographic assessment (WHO, 2003). The use of an MRI is not preferable, but it does have the advantage of providing some resolution of the internal structure of cancellous bone. Finally, peripheral DXA (pDXA) is widely used to measure BMD at the distal radius and calcaneus with high precision. It is also more portable and less expensive than the DXA, but it should not be used in diagnosing osteoporosis or predicting fractures. Its usefulness, therefore, is only in identifying patients that require further assessment (WHO, 2003). Also, peripheral QCT (pQCT) scans are available for the measurement of the forearm and have the ability to report a volumetric density of both the cortical and trabecular bone in the ultradistal radius (Fogelman & Blake, 2000). However, these other methods are not useful in reporting the effects of an intervention due to the small changes that occur in comparison to the spine and hip BMD.

Biochemical Markers

Biochemical markers that reflect the remodeling process are often used in order to provide more direct information. While DXA is very slow in revealing changes in bone density, biochemical markers respond more rapidly to interventions. Markers of bone metabolism are classified into three categories: enzymes or proteins secreted by cells

involved in the remodeling process, breakdown products generated in the resorption of old bone, and byproducts produced during bone formation. Due to the shorter duration of the resorption phase, these breakdown products of resorption respond quicker to changes in remodeling than do markers of bone formation (Vasikaran, 2008).

Markers of Bone Resorption

TRAP. Tartrate-resistant acid phosphatase (TRAP) is a lysosomal enzyme found in bone that can be measured in the serum or plasma by electrophoresis or immunoassay. However, serum concentrations are higher due to the release of acid phosphatase from erythrocytes during clotting (Watts, 1999). Two different isoforms of TRAP exist, a sialic acid-containing isoform, TRAP5a, and a nonsialylated isoform, TRAP5b. TRAP5a is derived from activated macrophages and has low specific activity. TRAP5b is a cleaved two-subunit form derived from osteoclasts as opposed to the non-cleaved TRAP5a. Therefore, an immunoassay that is specific for TRAP5b must be used to gain useful information about bone resorption (Nenonen et al., 2005). Studies observing elderly women have supported the usefulness of TRAP5b in fracture predictability (Gerdhem et al., 2004; Lenora, Ivaska, Obrant, & Gerdhem, 2007). Lenora and colleagues found that "S-TRAP5b was correlated to aBMD change in the legs, and after adjustment for baseline aBMD, to aBMD change at the femoral neck" (Lenora et al., p. 1302).

Pyridinium cross-links (pyridinoline and deoxypyridinoline). Collagen breakdown products include the cross-links pyridinoline (Pyd) and deoxypyridinoline (Dpd) that can be measured in urine by high-performance liquid chromatography (HPLC) or immunoassay. These cross-links are responsible for connecting strands of the type 1

collagen triple helix structure at the lysine or hydroxylysine residues and are released with the resorption of type 1 collagen. Pyd and Dpd are released from bone in about a 3:1 ratio (Eyre, 1987). However, there may be some error in measuring these peptides specifically for bone since Pyd is also found in articular cartilage and soft tissue. The proportion in bone is much greater with a greater turnover (Watts, 1999). Of all the cross-links released during resorption, 60% are bound to protein while the remaining 40% are free. Pyridinium cross-links are not metabolized or absorbed from the diet (Colwell, Russell, & Eastell, 1993; Watts). Deoxypyridinoline would be more applicable to measuring changes in bone resorption since it has only been found in type I collagen of the bone (Eyre). Urinary Dpd/creatinine has been found to be significantly higher (p = .027) in 75 year-old women who had recently sustained a fracture while other markers were elevated as well (Obrant et al., 2005). A disadvantage to measuring pyridinolines is the involvement of a complex and time-consuming process by HPLC. Free Dpd is insensitive to acute changes in osteoclast activity (Rubinacci, Melzi, Zampino, Soldarini & Villa, 1999), and the urinary excretion of Dpd is 50-70% higher at night than in the morning (Blumsohn et al., 1994; Nielsen, Brixen, & Mosėkilde, 1990).

Hydroxyproline. Hydroxyproline is another product of collagen breakdown excreted in the urine that can be measured by colorimetry or HPLC after hydrolysis to convert the peptide and polypeptide forms into free form (Watts, 1999). Hydroxyproline is formed by the posttranslational hydroxylation of proline, an amino acid that is prevalent in collagen. The liver catabolizes most free hydroxyproline liberated from the bone, but about 10% of this hydroxyproline is released in small polypeptide chains that are excreted in the urine.

However, hydroxyproline is also a product of the breakdown of complement, nonskeletal collagen, and procollagen extension peptides. About 50% of urinary hydroxyproline is derived from bone collagen breakdown (Deacon et al., 1997). Because nonskeletal collagen includes dietary collagen, diet may influence hydroxyproline levels. These levels may also be partly reflective of bone formation due to procollagen extension peptides being byproducts of bone formation.

Cross-linked telopeptides. Cross-linked telopeptides include N-telopeptides (NTx) and C-telopeptides (CTx), both of which are measured from the urine or serum by an enzyme-linked immunosorbent assay (ELISA). These cross-links are released from the collagen during bone resorption attached to amino-and carboxy-terminal fragments through the enzyme cathepsin K, a cysteine proteinase (Watts, 1999). Several studies have shown both CTx and NTx to be the best predictors of BMD, with serum CTx being a better predictor of BMD. Both CTx and NTx concentrations were significantly decreased with the ingestion of calcium-fortified spring water, although CTx demonstrated the most significant response (Guillemant, Accarie, de la Gueronniere, & Guillemant, 2003). When measured after 24 weeks of hormone replacement therapy, NTx was decreased by 39% while CTx decreased by 67% (Hannon, Blumsohn, Taylor, & Eastell, 1998). This difference between sensitivity could be due to different rates of clearance. Other factors that have resulted in decreases in CTx include oral glucose tolerance tests, fat and protein intakes, and normal meals (Bjarnason et al., 2002). CTx is subject to a diurnal rhythm with a peak during the night, but an acute fast has been found to diminish the circadium rhythm of CTx (Schlemmer & Hassager, 1999).

Markers of Bone Formation

Alkaline phosphatase. Alkaline phosphatase is an enzyme associated with the plasma membrane of osteoblasts, but it is found in the liver, intestine, and placenta as well (Moss, 1987). The bone and liver isoenzymes each contribute about 45% to the serum concentrations (Watts, 1999). Bone alkaline phosphatase (BAP) was found to be the best predictor of BMD reductions at the distal radium in one-third of diabetic hemodialysis patients with low parathyroid hormone (< 180 pg/ml). Those hemodialysis patients with BMD reductions also showed a tendency toward higher N-terminal propeptides of type I procollagen (PINP) and Pyd, but did not reach significance (Ueda et al, 2005). Bone alkaline phosphatase had the lowest intra-and inter- assay CVs (2.2% and 3.1%, respectively) of all biochemical markers (Kumeda et al., 2000). This is due to the stability of the BAP molecule. Serum BAP is a particularly useful marker in hemodialysis patients because it is not influenced by renal dysfunction because of its exclusive metabolism in the liver (Ueda et al., 2002; Ueda et al., 2005).

Osteocalcin. Osteocalcin is the major noncollagen protein of bone matrix comprised of 49 amino acids and was formerly known as the "GLA protein" because of its richness in glutamic acid. During matrix formation, a portion of intact osteocalcin is released into circulation and can be measured from plasma or serum by immunoassay as a marker of bone formation. Breakdown fragments of osteocalcin are also released during bone resorption and formation. Osteocalcin binds to lipids and therefore may be reduced in lipemic serum. Also, erythrocytes liberate proteolytic enzymes that degrade osteocalcin (Watts, 1999). Assays that use antibodies that recognize both intact osteocalcin and the

large N-terminal midmolecule fragment are the best clinical indicators (Menkes et al., 1993). Also, uncarboxylated osteocalcin may be a better predictor of fracture due to vitamin K's role in affecting the amount of osteocalcin carboxylation (Szulc, Chapuy, Meunier, & Delmas, 1993; Vergnaud et al., 1997). Osteocalcin, osteonectin, and osteopontin comprise 10% of the bone matrix.

Procollagen extension peptides. Type 1 collagen, which comprises 90% of the bone matrix (Watts, 1999), is synthesized form large procollagen molecules secreted from osteoblasts. These procollagen molecules undergo extracellular cleavage at the amino (PINP) and carboxy (PICP) termini, resulting in the release of amino and carboxy-terminal procollagen extension peptides (Scariano et al., 1998). Carboxy-terminal procollagen extension peptide is a globular protein of 1000 kDa that contains disulfide bonds while PINP is an elongated protein of 35 kDa (Watts). Both of these peptides may be incorporated into bone matrix, cleared by the liver, and measured by immunoassay. However, this marker may not be the best due to an increased turnover of nonskeletal collagen increasing the concentration of both peptides (Watts).

Timing of Changes in Biochemical Markers

The timing of blood samples is variable in the published literature when looking at the effect of an intervention on biochemical markers of bone turnover. Studies concerning the effect of exercise on biochemical markers can be divided into two categories: acute bouts of exercise and long-term studies. Another important difference that must be considered when talking about timing of blood samples is the type of exercise performed. Studies that measure the effect of resistance exercise are most likely

not going to have the same results as those concerning aerobic exercise. Other variations include the intensity and duration of exercise, fitness level and gender of participants, and the specific biomarkers that were measured.

Of the studies that measured the effect of acute bouts of aerobic exercise on markers of bone turnover, all of them showed some change in markers (Brahm, Piel-Aulin, & Ljunghall, 1997; Guillemant et al., 2004; Langberg, Skovgaard, Asp & Kjaer, 2000; Maimoun et al., 2005; and Zitterman et al., 2002). These studies varied in duration, timing of samples, and participant demographics.

Two studies tested the acute effects of aerobic exercise by means of the cycle ergometer on markers of bone turnover. Maimoun et al. (2005) had 7 male competitive road cyclists perform two separate 50 min steady state exercises tests on 2 days with 4-7 days between each test. One test was performed at 15% below the individual's ventilatory threshold while the other was performed at 15% above their ventilatory threshold (VT). Samples were taken at rest, at 30 min of exercise, at 50 min of exercise, and after 15 min of recovery. In the study by Guillemant et al. (2004), 12 young male triathletes performed a 60 min cycle ergometer exercise at 80% of their VO_2max. Blood samples were collected at every 30 min the hour before exercise, during the 60 min exercise, and during the 2 hr recovery period. Both studies found increases in CTx after 30 min of exercise. Maimoun et al. found an 11% increase in CTx at 30 min and 16% increase at 50 min in response to exercise performed at 15% above the VT. Like Maimoun et al., Guillemant et al. (2004) found an increase in CTx at 30 min after the onset of exercise. However, the studies differed in that Maimoun and colleagues found that CTx returned to initial values

after 15 min of recovery while the participants in the Guillemant et al. (2004) study had CTx values that remained elevated 2 hr following the exercise (45-50%). This response was suppressed with the ingestion of high-calcium mineral water. In addition, both Maimoun et al. and Guillemant et al. studies found significant increases in PTH. Maimoun et al. showed a 41% increase in PTH at 15% above the VT after 50 min of exercise and peaked during recovery (80%). Guillemant et al. (2004) found significant increases in PTH at 30 and 60 min of exercise but returned to baseline during recovery. The response of BAP to the cycle ergometer tests also differed between the two studies. Maimoun et al. found an increase in BAP after 30 min (12% for –VT and +VT) and 50 min (12% for –VT and 14% for +VT) while Guillemant et al. (2004) found no difference in BAP. Finally, Maimoun et al. found a significant increase in OC after 50 min +VT.

In contrast to the Maimoun et al. (2005) and Guillemant et al. (2004), 18 male athletes (triathletes, game sports, and track and field sports) showed a decrease in CTx that approached significance after performing a 60 min treadmill test at 70% of their maximal speed but returned to baseline at 3 hr post exercise (Zitterman et al, 2002). In addition, PICP (bone formation marker) values were significantly lower in response to exercise when compared to rest (-9.8%). PTH was not affected. These findings would suggest that CTx responds differently to treadmill testing that it does in response to the cycle ergometer.

Other studies measured the effects of an acute bout of running on markers of bone turnover (Brahm et al., 1997; Zitterman et al., 2002). Brahm and colleagues implemented a 35 min maximal treadmill test on 20 individuals (10 women and 10 men). They began

the protocol with a 10 min warm-up at 30% of VO_2max followed by 10 min each at 47% VO_2max and 76% VO_2max. The treatment ended with a 4-5 min maximal effort until exhaustion. Blood was drawn at rest, immediately after each submaximal interval, after the maximal exercise, and after 30 min and 24 hr of recovery. The results showed significant intensity-related increases in PICP, tALP, and PTH concentrations during exercise with PTH remaining elevated during recovery. The findings for PICP differed from those found by Zitterman et al. who found a decrease in PICP values. The increase in tALP is consistent with the increases found by Maimoun et al. (2005) in BAP. Finally, the increase in PTH is consistent with findings by Maimoun et al. and Guillemant et al. (2004).

The type and duration of aerobic activity may also affect the activity of markers of bone metabolism. Langberg et al. (2000) found a transient decrease in PICP immediately after a marathon in 7 healthy male runners that was followed by an increase in PICP in the days after the marathon and peaked at 72 hr postexercise. The PICP marker then returned to baseline 5 days following the marathon. In addition, plasma ICTP, a marker of collagen resorption, increased significantly in response to the marathon (measured immediately after the completion of the marathon) and returned to baseline 24 hr postexercise. These results would indicate that muscle breakdown was elevated following a marathon and peaked at 3 days post marathon. The increases in PICP are consistent with the findings of Brahm et al. (1997) but differed from that of Zitterman et al. (2002). Results from acute aerobic exercise studies are found in Appendix A.

Several studies also measured the acute effects of resistance exercise on markers of bone turnover (Ashizawa et al., 1998; Whipple et al., 2004). Ahizawa et al. measured the effects of a single bout of resistance exercise (3 sets of 10 repetitions with the first set at 60% 1RM and the 2nd and 3rd sets at 80% 1 RM) on calcium and bone metabolism in 14 Oriental untrained males. They performed seven exercises that included bench press, back press, arm curl, double leg extension, bent leg incline sit-up, lateral pull down, and leg press. Samples were taken at 1600 and 0800 hr on a control day, an exercise day, and 3 postexercise days. Urinary Dpd decreased on Days 1 and 3 postexercise. In addition, plasma PICP concentrations decreased slightly on the exercise day and decreased further the day following exercise. There was also a significant decrease in serum BAP 2 and 3 days after exercise. No significant changes were found in serum osteocalcin, but serum TRAP activity decreased slightly on the exercise day and decreased significantly the day after exercise. However, TRAP values returned to resting levels by the end of recovery. The study by Whipple and colleagues also utilized a resistance protocol that included 3 sets of 10 repetitions for seven exercises in 9 young healthy men. In contrast to Ashizawa et al., participants performed the sets at 50, 75, and 100% of the 10-repetition maximum, respectively. Both studies implemented bench press, leg press, lateral pull down, and arm curl. However, Whipple et al. included seated row, leg curl, and back extension in the protocol. Blood samples were taken immediately before, immediately after, and at 1, 8, 24, and 48 hr post exercise. Consistent with Ashizawa et al. finding a decrease in Dpd, a marker of bone resorption, Whipple et al found a significant decrease in serum NTx at 1, 8, and 48 hr post exercise. Consistent with findings by Maimoun et al. (2005) and Brahm

et al. (1997), BAP was significantly higher immediately following exercise. While PICP remained unchanged over time, the ratios of BAP: sNTx and PICP: sNTx were significantly higher at 1 and 8 hr postexercise when compared to preexercise values.

Finally, Tosun et al. (2006) conducted a study measuring the acute effects of a 30 min of brisk walking on a treadmill with and without carrying 5 kg of weight in a backpack on 9 healthy premenopausal women. Blood samples were collected at rest, the 30th min of exercise, after 15 min of recovery, and at 24 hr. Urine samples were taken at the 1st and 24th hr. Significant decreases in total ALP were found in the aerobic exercise group while significant increases were found in the weight-lifting group at the 24th hr. Aerobic exercise without weights resulted in a significant increase in PTH levels at the 30th min but returned to baseline after 15 min of recovery. The PTH concentrations with weight-lifting were insignificant.

Based on the above studies, the results of an investigation measuring the effects of a single bout of resistance exercise on biomarkers of metabolism may be greatly dependent on the time points that blood or urine samples were taken. In several studies involving acute bouts, significant results were found during or immediately after the exercise bout. While some studies mentioned have shown significant results at 24 hr post exercise or greater (Ashizawa et al., 1998; Brahm et al., 1997; Langberg et al., 2000; Tosun et al., 2006; Whipple et al., 2004), other studies had markers return to baseline within hours of recovery (Brahm et al.; Guillemant et al., 2004; Maimoun et al., 2005; Zitterman et al., 2002). The studies above would suggest that the recovery time needed for markers of bone metabolism to return to normal is dependent on the type, duration, and intensity of

exercise. In conclusion, weight-bearing exercise as well as long bouts of aerobic exercise may be more likely to result in marker changes that last 24 hr or greater when compared to short bouts of aerobic exercise and the timing that blood samples are taken should be adjusted accordingly.

Ability of Markers to Predict Risk of Fracture

Many studies have demonstrated the ability of various markers of bone metabolism to predict fracture (Vasikaran, 2008). In diabetic patients on hemodialysis, bone alkaline phosphatase was significantly higher in those with reduced bone mineral densities. There was also an increase in PINP and Pyd although not significant (Ueda et al., 2005). In elderly women (75 years of age), those with a recently sustained fracture had increased levels of bone markers, especially serum TRAP5b and urinary LongOC/creatinine, the latter of which refers to only the longest urine OC fragment. Obrant et al. (2005) also used a separate assay for U-MidOC/crea which measured all the predominant urine OC fragments. The same study showed that women with low current physical activity had elevated levels of U-Dpd/creatinine and U-OC (Obrant et al.). In the same population, all bone markers were increased in women with vertebral fractures (S-BAP, 4 forms of S-OC, S-CTx, TRAP5b, U-Dpd), but were not significantly elevated with hip fracture. S-TRAP5b and one U-OC predicted the occurrence of fractures of any type while S-TRAP5b, the two U-OCs, and S-CTx predicted vertebral fracture (Gerdhem et al., 2004). Garnero, Sornay-Rendu, Claustrat, & Delmas (2000) found that "women with levels of BAP above the premenopausal range were at higher risk for fracture with a relative risk (RR) of 2.4" (Garnero et al., 2000, p. 1532). They also found an increased but not

significant risk of fracture in those that had increased levels of osteocalcin and propeptides of type 1 collagen (Garnero et al., 2000). In patients with osteoporosis being treated with raloxifene over 3 years, the percent change in OC was more reliable than the percent change in the femoral neck BMD in predicting the reduction of vertebral fracture risk (Sarkar et al., 2004). In a study with women in the early period of menopause treated with hormone replacement therapy or calcium supplementation, baseline urinary NTx and serum OC were found to be the most sensitive predictors of changes in spine BMD after 1 year. Also, the percent change in NTx and OC from baseline to 6 months best predicted bone gain or loss (Rosen, Chesnut, & Mallinak, 1997).

Although several bone markers have been identified that have the potential to predict fractures, their ability to predict the rate of bone loss after menopause is questionable. After studying 60 postmenopausal women ages 49-62, Rogers, Hannon, and Eastell (2000) found that there is a strong relationship between rates of spinal bone loss and levels of bone turnover markers, but they are not useful in predicting the rate of bone loss in an individual. Part of this is due to an exponential relationship between the rate of bone loss and the years since menopause, with the highest rate being in the early period. They also found a significant correlation between levels of NTx, immunoreactive free deoxypyridinoline (iFDpd), and PINP and the number of years since menopause (Rogers et al.).

Problems with Markers

The problems with biochemical markers of bone metabolism lie mostly with their variability. Also, not all biochemical markers are subject to the same rhythm and not all

individuals have the same rate of remodeling. Variations include changes due to diurnal rhythms, phase of the menstrual cycle, and season of the year. Studies have found changes due to bed rest, exercise, diet extremes, and other factors that could alter the remodeling process (Seibel, 2005). Dpd excretion is increased at night while diurnal variation is less of a factor in BAP and OC. There is also a day-to-day variation of about 10% for markers of bone formation and 20% for markers of bone resorption. Woitge et al. (1998) reported that values in the winter can be up to 12% higher than in the summer. Marker concentrations are also increased during puberty, after menopause, after fracture, with prolonged bed rest, and with weightlessness (Watts, 1999). The pattern of recovery after increases is also variable depending on the marker. Secondly, markers are not entirely bone-specific. For example, alkaline phosphatase can be derived from nonskeletal sources, and osteocalcin fragments may be the result of both bone resorption and formation (Watts). The third obstacle with markers is that the fluctuation is highly dependent on clearance from organs such as the clearance of BAP from the liver (BAP) and NTx and CTx from the kidney (Colwell & Eastell, 1996). Finally, changes in the rate of remodeling have a quicker effect on markers of bone resorption than formation due to the shorter length of time required for bone resorption (Watts).

Nutrition Influences on Bone Health

Calcium

With the majority of the calcium in the human residing in the skeleton (99%), it is no wonder that an adequate intake of calcium is essential in maximizing peak bone mass early in life as well as preventing bone loss, and maintaining the balance between bone

formation and resorption with age. For premenopausal women, the recommended dietary allowance (RDA) for calcium is 1000 mg/day. However, intakes above the recommended levels may result in additional benefits in total body BMC (Teagarden et al., 1998). The extracellular calcium concentration is regulated by a dynamic equilibrium between the intestine, kidney, and bone (WHO, 2003). The total amount of calcium absorbed is a combination of dietary calcium and extracellular calcium that has diffused in the lumen.

Calcium excretion has been measured by several investigators in order to determine the effect of different interventions on calcium balance and bone metabolism. The thought behind this is that during a steady state, the amount of calcium excreted in the urine corresponds to net calcium fluxes that enter the extracellular compartments from the intestine and bone. In the kidney, 98% of the calcium filtered is reabsorbed (WHO, 2003). Therefore, an increase in urinary calcium excretion could be a result of increased calcium mobilization from the skeleton or increased intake. Of studies that have investigated the effect of calcium supplementation on bone mass, adolescent individuals and post-menopausal women seem to benefit the most while it has not been shown to play a substantial role in premenopausal women (Devine, Dick, Heal, Cradle, & Price, 1997; Khan et al., 2001).

Because of the roles that dietary calcium and weight-bearing physical activity have on bone health, several studies have been performed to investigate the presence of an exercise-calcium interaction. These studies suggest that the beneficial effects of physical activity interventions are only apparent at calcium intakes greater than 1000 mg/day (Specked, 1996; Specked & Binkley, 2003). Further studies have found that this exercise-

calcium interaction is due to the general effect calcium has on bone sites compared with the region-specific effect that weight-bearing exercise has on the loaded site (Iuliano-Burns, Saxon, Naughton, Gibbons, & Bass, 2003; Uusi-Rasi, Sievanen, Pasanen, Oja, & Vuoiri, 2002). However, further studies are needed to illuminate the mechanism of action for the synergistic effects of calcium consumption and physical activity.

Vitamin K

Vitamin K is a vitamin that is most commonly consumed in the diet in the form of phylloquinone (vitamin K_1) which is found in high concentrations in green, leafy vegetables and in some plant oils (Kalkwarf, Khoury, Bean, & Elliot, 2004). The current recommendations for adequate intakes are 55-75 µg/d for children 4-18 yr of age and 90-120 µg/d for adults (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2000). Vitamin K serves as a co-factor of γ-carboxylase that's required to convert certain peptide-bound glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues in target proteins (Gla proteins) such as osteocalcin, the most abundant Gla protein contained in the bone matrix (Bolton-Smith et al., 2007; Iwamoto, Sato, Takeda, & Matsumoto, 2009).

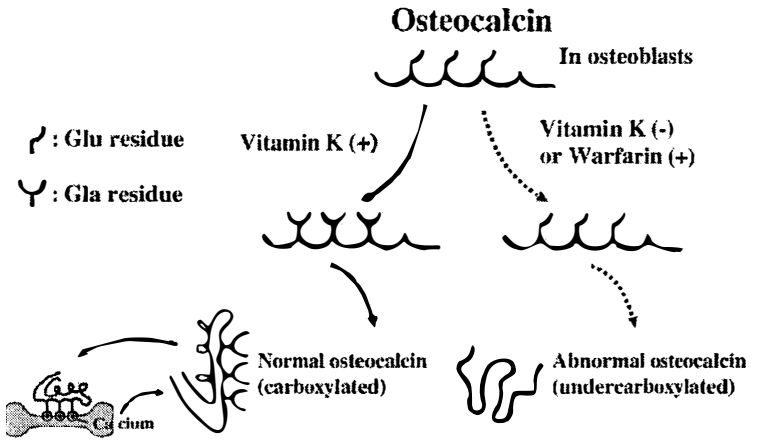

Figure 1.γ-Carboxylation of OC by vitamin K (Iwamoto et al.)

An inadequate intake of vitamin K results in a larger percentage of undercarboxylated osteocalcin (Binkley, Krueger, Engelke, Foley, & Suttie, 2000; Sokoll et al., 1997). Studies have shown that high circulating levels of undercarboxylated osteocalcin (ucOC) is an independent predictor of the risk of bone fractures and low BMD (Bolton-Smith et al., Kalkwarf et al.). However, other studies have found no affect on vitamin K_1 supplementation on bone turnover (Binkley et al.; Bugel et al., 2007; Kruger et al., 2006) or on regional BMD (Volpe, Leung, & Giordano, 2008). Further research is needed to determine the effects of an inadequate vitamin K intake on bone turnover and BMD.

Phosphorus

While phosphorus is an essential element to bone formation, there is more evidence surrounding the adverse effects of high intakes of dietary phosphorus on bone health. The RDA for phosphorus for adults is 700 mg/day. However, the calcium to phosphorus ratio is a greater determinant of bone health than is phosphorus intake alone. Low calcium

intakes as well as low calcium to phosphorus ratios have been shown to increase levels of PTH in young adults (Barger-Lux, Heaney, Lanspa, Healy, & DeLuca, 1995; Calvo et al., 1990; Palacios, 2006). Teegarden et al. (1998) reported that a 1:1 ratio of calcium to phosphorus was not optimal to maximize peak bone mass. Instead, they found ratios of 1:2 and 1:4 to further increase the total-body BMC by 5 or 7.4%, respectively (Teegarden et al.). They also found that increasing phosphorus intake in the presence of low calcium intake (600 mg/d) has a positive effect on both total-body and spine BMD and BMC, but increasing phosphorus intake (1800 mg/d) after calcium intake increases (up to 1400 mg/d) has a negative effect on total-body and spine BMD and BMC. However, other studies have reported that doubling the amount of phosphorus consumed has no effect on bone turnover (Bizik, Ding, & Cerlewski, 1996; De Laet, van Hout, Burger, Hofman, & Pols, 1997).

Protein

Protein exists as an essential component of bone formation, making up 22% of the bone tissue's weight (Dawson-Hughes, 2003) as well as playing a major role in stimulating the production of hormones and growth factors, such as IGF-I, that aid in bone formation (Palacios, 2006). Decades of research have demonstrated the presence of a positive relationship between protein intake and urinary calcium excretion (Kerstetter, O'Brien, & Insogna, 2003). Further studies have found that high intakes of dietary protein (> 1.5 g PRO/kg) result in hypercalciuria most likely due to increased calcium absorption while low protein intakes (≤ 0.8 g PRO/kg) result in increased PTH and calcitriol as well as reduced calcium absorption (Kerstetter, O'Brien, & Insogna, 1998;

Kerstetter, O'Brien, & Insogna, 2003). A study by Roughead, Johnson, Lykken, & Hunt (2003) used radiotracers to measure the effect of high and low meat diets over 8 weeks in postmenopausal women. The study found no differences between calcium retention and biomarkers of bone metabolism among the two groups. Although past studies have linked hip fractures with high protein intakes (Abelow, Holford, & Insogna., 1992), more recent studies have found that it is the ratio of calcium to protein intakes as well as the intake of phosphorus that influences bone health (Teegarden et al., 1998; Weaver et al., 1999). Teegarden and colleagues found high or low protein intakes in relation to calcium to have similar effects on total-body and spinal BMD and BMC as that of phosphorus and calcium. To further defend these findings, phosphorus and protein were both found to be negatively associated with radial bone mass in young women while the ratio of calcium to phosphorus and calcium to protein were positively associated with radial bone mass (Metz, Anderson, & Gallagher Jr., 1993). Weaver et al. (1999) calculated that an increase of dietary calcium intake to about 1200 mg/day would offset the losses caused by a phosphorus intake of 1000 mg/d and a protein intake of 76 g/d. Adequate consumption of dietary protein may provide further benefits to bone health that include aiding in the healing of fractures, preventing bone loss after fracture, improving serum prealbumin levels, increasing IGF-I, and decreasing the length of rehabilitation in patients with an osteoporotic hip fracture (Schurch et al., 1998). Dawson-Hughes (2003) suggests that an adequate intake of calcium may help facilitate this favorable effect of protein on the skeleton.

There has also been an increased amount of attention on the effect of protein supplementation in athletes concerned with performance. Ballard, Clapper, Specker, Binkley, & Vukovich (2005) investigated the role of protein supplementation during six months of a strength and conditioning program on bone metabolism. All subjects were between 18 and 25 years of age and performed strength training and running on alternate days with the running lasting 45 min per session. One group took a protein supplement twice daily while the other group took an isocaloric carbohydrate supplement twice daily. The average protein intake between the two groups was about 2.2 g/kg body weight/d for the protein group and 1.1 g/kg body weight/d for the carbohydrate group. They found that there was a greater increase in plasma IGF-I, serum BAP, and urinary NTx in the protein group than the carbohydrate group. However, urinary NTx concentrations decreased in both groups at six months while serum BAP continued to increase. Men also had a higher IGF-I in the study than women (Ballard et al., 2005). Kraemer et al. (1998) confirmed these results with an increase in IGF-I concentrations after a protein or carbohydrate supplement was added to the normal diet during a resistance-training program. In conclusion, studies suggest that an adequate or increased level of protein along with appropriate levels of calcium consumption and exercise promote osteoblast-mediated bone formation. While the current recommendations for protein are 0.8 g/kg body weight/d, slightly higher amounts may be more beneficial for bone health.

Vitamin D

Vitamin D exists in two forms, cholecalciferol and ergocalciferol. Cholecalciferol (vitamin D_3) is generated in the skin while ergocalciferol (vitamin D_2) refers to the plant

form of the vitamin. Ultraviolet light is responsible for the conversion of 7-dehydrocholesterol to cholecalciferol in the skin. It is then converted to 25-hydroxycholecalciferol by 25-hydroxylase in the liver and finally to its active form, 1, 25-dihydroxycholecalciferol, by 1α-hydroxylase in the kidney. The active form of the vitamin functions as a hormone and is required for calcium absorption in the lumen as well as for phosphate and magnesium absorption. Most people only require 15-30 min of sunlight exposure per day for adequate levels of vitamin D synthesis (Reid, Gallagher, & Bosworth, 1986). Because the synthesis of cholecalciferol is dependent on UVB rays, certain populations should consume fortified foods or other foods that contain vitamin D to avoid deficiency. Vitamin D deficiency is characterized by rickets in children and osteomalacia in adults and is usually accompanied by elevated levels of PTH. Deficiency is most common in elderly individuals due to decreased exposure to sunlight and decreased ability to synthesize vitamin D and convert it to its active form. This problem is more common in individuals living in latitudes above 40 °N or below 40 °S due to no cutaneous production during the winter months (Webb, Kline, & Holick, 1988) and in those with a darker skin pigmentation. Those at risk for vitamin D deficiency should consume a supplement between 400 and 800 IU/day in order to reduce PTH concentrations and increase bone density, especially at the femoral neck (WHO, 2003; Reid et al., 1986). While vitamin D status does effect bone health, Barnes, Robson, Bonham, Strain & Wallace (2006) found that eight weeks of supplementation resulted in no significant changes in biomarkers of bone metabolism (PTH, CTx, BAP) in young adults in Northern Ireland.

Magnesium

Being a cofactor for over 300 enzymes, magnesium is required for an extensive array of metabolic functions. Magnesium's role in the bone is somewhat magnified with the bone containing about 60% of all the body's magnesium (Palacios, 2006). One of its more important roles is reducing the size of hydroxyapatite crystals, and preventing the bones from becoming brittle. Depletion of magnesium in the bone may result in increased inflammatory cytokines, increased osteoclastic bone resorption, and impaired bone growth (Rude, Gruber, Wei, Frausto, & Mills, 2003). Magnesium deficiency has also been shown to result in altered calcium balance (Fatemi, Ryzen, Flores, Endres, & Rude, 1991), while magnesium supplementation has resulted in increased BMD after 1-2 years in menopausal women (Sojka & Weaver, 1995). The current RDA for magnesium is set at 400 mg/day for individuals between 19 and 30 years of age. For those over 30, the RDA is 320 mg/day for females and 420 mg/day for males.

Other Nutrients

Fluoride is most commonly recognized for its role in preventing dental carries, but high doses of fluoride may have a detrimental effect on bone health. The osteoblastic stimulating effect of fluoride has increased interest in using pharmacological doses in treatment of osteoporosis. High doses may result in the widening of bones and reduced BMD in cortical regions of the skeleton due to increased calcium excretion (WHO, 1990) whereas lower levels of fluoride (11-20 mg/day) have resulted in decreased vertebral fracture risk as well as increases in the BMD of the lumbar spine and femoral neck

(Palacios, 2006; Reginster et al., 1998; Ringe, Kipshoven, Coster, & Umbach, 1999). The adequate intake (AI) of fluoride lies at 3 mg/day for women and 4 mg/day for men.

Zinc is an essential nutrient for osteoblastic activity, collagen synthesis, and alkaline phosphatase activity (Palacios, 2006). Increased levels of zinc in the urine have been associated with total body BMC in women with postmenopausal osteoporosis (Relea et al., 1995) as well as with hydroxyproline levels (Foldes et al., 1987). The current RDA for zinc is 8 mg/day in women and 11 mg/day in men although an intake of 15 mg/day has been associated with increases in BMD in postmenopausal women (Strause, Saltman, Smith, Bracker, & Andon, 1994).

The upper limit (UL) set for boron is 20 mg/day while no recommended levels of intake have been set. A boron intake of about 17 mg/day has been associated with gains in bone mass (Beattie & Peace, 1993; Palacios, 2006). Other functions of boron related to bone include reductions in calcium and magnesium excretion, increased serum levels of estradiol, and increased calcium absorption (Beattie & Peace; Palacios).

Copper plays a large role in forming strong, flexible connective tissue through the actions of lysyl oxidase, a copper-containing enzyme that is essential in the cross-linking of collagen fibrils (Palacios, 2006). Copper supplementation has been associated with reduced bone loss in both peri- and postmenopausal women. While the RDA for copper is 900 mg/day for adults, levels of 2.5-3 mg/day have been associated with reduced bone loss and an increased BMD in both pre and postmenopausal women (Palacios; Strause et al., 1994).

Manganese is a cofactor for several enzymes with an adequate intake (AI) level of 1.8 mg/day in women and 2.3 mg/day in men. However, levels above the AI up to 5 mg/day are associated with increases in BMD (Palacios, 2006; Strause et al., 1994). Potassium recommendations of 4700 mg/day for adults result in a greater trochanter BMD (Tucker et al., 1999), improved calcium balance, decreased bone resorption, increased bone formation, and improved BMD in the hip and forearm (Lemann, Pleuss, & Gray, 1993; Palacios; Tucker et al., 1999).

Many other nutrients play a role in bone formation due to their role as a cofactor. Iron is a cofactor for 25-hydroxycholcalciferol hydroxylase, an enzyme required in vitamin D synthesis, as well as in prolyl and lysyl hydroxylases, enzymes involved in collagen bone matrix synthesis (Palacios, 2006). Vitamin C is a cofactor in the hydroxylation of lysine and proline, which are required amino acids in the cross-linking of collagen fibrils in bone. Vitamin C also stimulates alkaline phosphatase activity (Palacios). Intakes higher than the RDA of 75 mg/day in women and 90 mg/day in men have been related to better bone health (Morton, Barrett-Connor, and Schneider, 2001). Vitamin A, with a RDA of 2333 IU for women and 3000 IU for men, plays a substantial role in the bone remodeling process. Both osteoblasts and osteoclasts have receptors for retinoic acid, and either high or low intakes of vitamin A can result in an increased risk of fracture. Vitamin B_{12} acts as a cofactor on bone alkaline phosphatase and osteocalcin, and several studies have shown that vitamin B_{12} deficiency results in an increased risk of fracture (Tucker et al., 2005). Other nutrients that have an indirect effect on bone health include riboflavin, niacin, vitamin B_6, folic acid, and thiamin (Palacios).

Because of the effects that a variety of nutrients have on bone metabolism, diets in an intervention should be monitored to prevent deficiency or toxicity from being a confounding factor in study results.

The Effect of Aerobic Exercise on Bone Metabolism

Aerobic Exercise

The affect of distance running on BMD at different sites has been assessed by several studies. Nevill, Burrows, Holder, Bird, & Simpson (2003) found that the legs have a significantly greater BMD in eumenorrheic female runners than at all other sites after a 10-site assessment in adult female runners. However, this result should be taken under careful consideration due to the fact that bones at different sites are also shaped differently. The same study also found that upper body sites including the arms, ribs, and thoracic spine had the lowest mean BMD when compared to other skeletal sites. The greater BMD value at the hip than the spine in runners is explained by the greater magnitude of loading at the hip (Hind et al., 2006) However, men and women runners that included two resistance-training sessions per week into their regimen had a greater lumbar spine (Hind et al.). The lower body measurements that were found to be impacted the most by running were the legs and hip. These results suggest that BMD is the greatest at the point that is subjected to the greatest mechanical loading. Runners with greater body mass index (BMI) were also found to have an increased BMD, most likely due to increased mechanical loads (Tomkinson et al., 2003). This theory is not limited to distance running. Tosun et al., (2006) found that a single bout brisk walking for 30 min had a stimulating effect on bone turnover (decrease in ALP after 24 hr of recovery and

increase in PTH levels at the 30th min). However, a significant increase in ALP but not PTH was seen when the same subjects performed brisk walking while carrying 5 kg of weight for 30 min in a backpack (Tosun et al., 2006). This further emphasizes the beneficial effects of mechanical loading on bone remodeling. Because increases in BMD are associated with mechanical loading, exercises such as cycling and swimming do not produce the same results as weight-bearing exercises.

Effect of Mileage on BMD

After comparing adult female runners from the ages of 18 to 44 yrs, Nevill et al. (2003) concluded that running greater distances results in a positive osteogenic effect. However, the effect of mileage varied significantly between sites with the BMC of the legs and arms benefiting the most. Tomkinson et al. (2003) found an adverse effect on BMD of high mileage in postmenopausal women who were not on hormone replacement therapy (HRT). However, the same study found no bone loss in premenopausal women. Other studies have also reported an inverse relationship between the distance run per week and the BMD of the lumbar spine (Hind et al., 2006). Also, there was found to be a negative correlation between the years of training and overall BMC (Nevill et al.). This affect varied across different sites, with the rate of decline in BMC being the lowest in the legs. This negative association may be a reflection of lighter training loads. A study by Kaga et al. (2004) comparing high school and adult runners found the total BMD and leg BMD to be significantly higher in adult athletes. However, the lumbar BMD of the high school runners was significantly higher than in adults. The study concluded that long-distance training over a long period of time could be beneficial to cortical bone, but

the effect is enhanced only during eumenorrheic states. Because the adults had both significantly higher mean monthly running distances and athletic careers, further research should be performed to determine the role of distance versus years of training on BMD (Kaga et al.).

Menstrual State, Estrogen Levels, and Oral Contraceptives on BMD

When studying the effects of bone formation and resorption markers in premenopausal women, the phase of the menstrual cycle during which blood samples are taken must be duly noted. A study by Chiu and colleagues (1999) showed that levels of serum Dpd were higher during the follicular phase than in the luteal phase. Serum Dpd was also negatively correlated with serum estradiol levels measured 6-8 days earlier and with progesterone measured concurrently as well as 2, 4, and 6 days earlier. Bone alkaline phosphatase was found to be negatively associated with estrogen measured 6 days earlier and positively associated with concurrent serum Dpd. However, serum BAP, OC, and urine Dpd levels did not significantly change across the menstrual cycle (Chiu et al.). Zittermann et al. (2000) found that urinary Pyd and Dpd concentrations were lowest 3 days after ovulation and highest during the early follicular phase. They also found a negative but nonlinear correlation between serum concentrations of estradiol and renal Dpd and Pyd (Zittermann et al., 2000). Higher mean serum OC levels were found concurrently with higher mean urine Dpd levels and showed a trend toward lower hip BMD. Chiu et al. concluded that low levels of estrogen and progesterone during the follicular phase were associated with increased bone resorption. This is in agreement with Zittermann et al. (2000) in their findings that PICP levels were the lowest and Pyd and Dpd levels were highest with low

estradiol levels during the early follicular phase of the menstrual cycle (Zittermann et al., 2000). It is thought that PICP increases during the early follicular phase may be due to a direct estrogen effect on bone formation. This theory is based on the fact that osteoblasts exhibit estrogen receptors, and estrogens have been found to enhance collagen synthesis (Eriksen, Colvard, & Berg, 1988; Komm, Terpening, & Benz, 1988; Zittermann et. al, 2000). In addition, Gorai, Chaki, Nakayama, and Minaguchi (1995) found NTX to be highest at mid-cycle and lower again during the luteal phase.

The onset of menopause has resulted in changes in biochemical markers of bone metabolism associated with age as well as hormone status. Chiu et al. (1999) and Kushida, Takahashi, Kawana, & Inoue (1995) both found that OC increases with age. In addition, Kushida et al. reported increases in alkaline phosphatase, PICP, Pyd, and Dpd with age as well as with the onset of menopause. ICTP, however, did not follow this pattern (Kushida et al.). In the same study, markers of bone resorption were much higher than that of bone formation in osteoporotic patients, and ICTP, Dpd, and Pyd were found to be the most useful in evaluating vertebral osteoporosis (Kushida et al.). A study by Tomkinson et al. (2003) assessed the changes in bone mineral density in the hip and spine before, during, and after menopause in elite runners. This was a follow-up study consisting of 35 of the original 75 participants. For postmenopausal women, those on hormone replacement therapy (HRT) during the study were found to maintain bone density while those who were not on HRT or ceased taking it lost bone density, with the least amount lost at the femoral trochanter. This is most likely caused by the femoral trochanter being the site at which the most direct muscular loading takes place during

running. The study concluded that HRT in combination with exercise in post-menopausal women increased BMD more than either alone. A study by Kaga et al. (2004) found high school athletes with regular menstruation to have higher levels of bone density than in those that had irregular or absent menstruation. In comparison with adult runners, long-distance training had the most deleterious effect on bone density in high school athletes with irregular or absent menstruation due to disturbances in pubertal sex hormone secretion (Kaga et al.). Therefore, bone density is the greatest in those athletes with regular menstruation and estrogen levels.

Oral contraceptives have been shown to cause a decrease in BMD in several studies. A study by Ott and colleagues (2001) investigated the effects of depot medroxyprogesterone acetate (DMPA) contraception and oral contraceptives on bone metabolism. They found that women using DMPA have higher markers of bone resorption (NTx) and oral estrogen-progestin contraception users had lower markers of both bone resorption and bone formation (OC) than women not using hormonal contraception. However, the decrease in markers of bone formation and resorption in oral contraceptive users was not accompanied by either a decrease or increase in bone density (Ott et al.). In contrast, Recker et al. (1992) found an increase in bone density in oral contraceptive users compared to nonusers among college-aged women. In the study by Ott et al., college aged women showed a positive correlation between BMD of the spine and the duration of oral contraceptive use, but no positive association was found at the hip. Garnero and colleagues (1995) were in agreement with Ott et al. (2001) in that oral contraception was associated with a significant decrease in bone turnover. They also

suggested that the decrease in bone turnover might have a beneficial effect on BMD after prolonged use (Garnero, Sornay-Rendu, & Delmas, 1995). This finding is contradicted by lower lumbar spine T-scores in women runners taking oral contraceptives (Hind et al., 2006). Weaver et al. (2001) found similar results with an interaction between exercise and oral contraceptive use resulting in a decrease in spinal BMD and BMC during the first 6 months of the intervention and remaining lower for the entire 2 years of the study (Weaver et al., 2001). However, another study found that female athletes taking oral contraceptives had an increase in BMD, with the greatest increases being in those with the lowest BMD at baseline which included athletes with menstrual disturbances (Rickenlund et al., 2004). While there is conflicting evidence concerning the effects of oral contraceptive use on BMD, their possible role in preventing bone loss or increasing BMD should not be ruled out.

The Effect of Resistance Training on Bone Metabolism

It has been suggested that the optimal stimuli for an increase in BMD is exercise that results in high rates of strain on bones. This includes exercises that require heavy loading with few repetitions (Fujimura et al., 1997). These high rates of strain would result in a positive and site-specific impact on bone mass. However, the mechanism for this action is still being investigated. The site-specific response of bone to exercise is explained by Wolffe's law which states that "bone responds to the forces placed upon it, such that BMD is greatest in regions of high force application (Winters-Stone & Snow, 2006, p. 1203)." Winters-Stone and Snow's study with premenopausal women reinforced the contents of this law by finding that the BMD of the spine was only significantly increased

compared to the control group in those that performed upper and lower-body resistance exercise, but not with lower-body alone after 12 months of exercise (three times per week). Upper body exercises alone were not performed. Furthermore, both resistance exercise groups showed significantly greater BMD in the greater trochanter compared to controls, but no difference between exercise groups (Winters-Stone & Snow). In addition, a study in female weightlifters found that "weightlifters had denser trabecular tissue at skeletal sites that are largely subjected to axial loading (in general, long bone ends) while the sites typically exposed to bending loads (i.e., forearm diaphysis) were larger compared with controls" (Heinonen et al., 2002, p. 472).

A study by Whipple et al. (2004) determined the effects of a single bout of resistance exercise on markers of bone metabolism in men immediately following exercise as well as at 1, 8, 24, and 48 hr postexercise. They found that exercise resulted in a significant increase in the ratio of bone formation markers to bone resorption 8 hr postexercise. BAP was significantly higher postexercise bout while PICP did not differ between exercise and control trials. However, serum markers of bone formation showed no change over time. The increase in ratio was explained by significantly lower serum NTx concentrations at 1 and 8 hr postexercise. However, marker concentrations returned to baseline within 24 hr of the exercise bout, suggesting that a single bout of resistance exercise acutely reduces bone resorption. When total ALP was measured post exercise bout, increases in ALP values with resistance exercise was detected at the 24th hour (Tosun et al., 2006).

A study by Menkes et al. (1993) found that 12 weeks of training in middle-aged men resulted in a 3.8% increase in the femoral neck along with a 19% increase in osteocalcin.

This increase in osteocalcin remained elevated through the 16th week of training along with a 26% increase in bone-specific alkaline phosphatase. A bed-rest study conducted by researchers at NASA/Johnson Space Center found that compared to controls, resistance training resulted in a positive effect on bone mineral density over a 17-week training period (Shackelford et al., 2004). While bone resorption markers were significantly increased during the study (NTx, Dpd, and Pyd), the difference between exercise and control groups was not significant. However, the difference was significant for markers of bone formation, which were increased for the exercise group but showed no change in the control group. Bone-specific alkaline phosphatase increased by 64% while osteocalcin increased by 31%. Also, BMD in the lumbar spine increased significantly (3%) from baseline in the exercise group as assessed by DXA. The study used both male and female subjects (Shackelford et al.). These results helped confirm results by Fujimura et al. (1997) that found that a weight training program performed three times per week for 4 months by young adult Oriental males resulted in significantly increased levels of osteocalcin and BAP and decreased urinary Dpd, all of which occurred during the 1st month of training with samples being collected 16 hr after exercise. No significant changes in BMD as measured by DXA were reported.

A study by Woitge et al. (1998) compared the effects of aerobic and anaerobic exercise on biomarkers of bone metabolism. They found that aerobic exercise favored bone formation by decreasing the concentrations of urinary cross-link excretion while not changing markers of bone formation. In contrast, anaerobic exercise over 8 weeks increased levels of BAP, OC, and Pyd. A study by Vincent and Braith (2000)

investigated the effect of the intensity of weight training on BMD in elderly men and women. They found that a higher intensity (80% 1-RM) and less repetition for three times per week over 24 weeks resulted in an increased BMD at the femoral neck (1.96%) as opposed to any change in BMD in the low-intensity change as assessed by DXA. Also, the high-intensity group increased osteocalcin by 39% and BAP by 7.1% as opposed to a 25% increase in osteocalcin by the low-intensity group. These studies suggest that while aerobic exercise suppresses the action of markers of bone resorption, resistance exercise works by increasing the levels of markers of bone formation and possibly suppressing the expression of markers of bone resorption. Vincent and Braith also demonstrated that high-intensity resistance training has significantly greater effects than low-intensity training on BMD and markers of bone formation and resorption.

Types of Exercises and BMD

Vincent and Braith (2002) found that the leg press, overhead press, and lumbar extensions had the greatest influence on both site-specific and total body BMD. Winters-Stone and Snow (2006) found significant increases in the spine after a series of upper-body resistance exercises that included upright row, one-arm row, latissimus dorsi pull-down, chest press, chest fly, biceps curl and triceps extension (3 sets of 8-12 repetitions with 1 min rest between sets). Lower-body resistance exercises that resulted in increased BMD of the greater trochanter included 100 jumps per session, squats, lunges, and calf raises (Winters-Stone & Snow). A rat study mimicking a squat movement while in hind leg suspension prevented bone loss in the distal femur (Fluckey et al., 2002). These results confirmed a study by Westerlind and colleagues that found that rats performing

hind limb "squats" with weighted vests had a significantly larger cancellous bone area in the proximal tibial metaphysic than controls (Westerlind et al, 1998). However, others have found that isokinetic exercises, independent of the mode, result in greater BMD increases in the upper limbs than in the lower (Nickols-Richardson, Miller, Wootten, Ramp, & Herbert, 2007). Nickols-Richardson and colleagues have concluded that it is the intensity of the exercise and not the mode that results in favorable changes in BMC and BMD (Nickols-Richardson, et al.).

Volume of Exercise and BMD

The volume of resistance exercise required to produce the greatest benefits on bone health has yet to be defined, and no studies to date have investigated the effects of the volume of resistance exercise on BMD in premenopausal women. Rubin & Lanyon (1984) found that the magnitude or rate of the strain induced in animal models was more important than the number of loading cycles. Turner and Robling (2003) identified 20 loading cycles to be the threshold before the desensitizing effect of repetitive loading occurred. If muscular strength was a predictor of BMD, it could be hypothesized that greater volumes of resistance exercise would result in a greater increase in BMD. Many studies measuring the effect of volume on strength have found that multiple sets of high-intensity resistance training result in significantly higher improvements than 1-set protocols (Borst et al, 2001; Humburg, Baars, Schroder, Reer, & Braumann, 2007; Kramer et al., 1997; Rhea, Alvar, Ball, & Burkett, 2002; Schlumberger, Stec, & Schmidteleicher, 2001). Interestingly, Humburg and colleagues found a significant difference between the 3-set and 1-set group's 1RM in the upper-body exercises but not

the lower body. Although the difference did not reach significance, they did find greater strength improvements (4.6 and 6.1% in the right and left leg press, respectively) in the 3-set group than the 1-set group (Humburg et al.). Fujimura et al. (1997) found that two sets of 10 repetitions of 7 to 8 exercises for the 1st month and three sets for the next 3 months (60% of 1 RM max for the first set and 80% of 1 RM max for the second and third sets) resulted in increases in osteocalcin and serum BAP (markers of bone formation) and a transient suppression of urinary Dpd (marker of bone resorption). However, further studies are needed to see the acute effects of volume in the premenopausal population.

Intensity of Exercise

Several studies have investigated the effect of intensity of training on BMD and markers of bone metabolism, although the absolute threshold for bone stimulation has yet to be determined. Vincent and Braith (2002) demonstrated that high-intensity training (80% 1RM) has significantly greater effects on BMD and biochemical markers than does low-intensity resistance training (50% 1RM). A study in male cyclists investigating the effects of two, 50 min cycling tests, one at 15% above and the other 15% below the ventilatory threshold, found that only the high-intensity exercise stimulated bone turnover and iPTH secretion (Maimoun et al., 2005). These effects were transient with markers returning to baseline within 24 hr of exercise (OC, CTx, and BAP). This study also raised the question of whether there is an inter-relationship between exercise duration and intensity since a study by Kristofferson and colleagues (1995) investigating the effects of short-term maximal exercise found no such rise in ionized PTH (Kristofferson, 1995). In contrast to these findings, moderate intensity resistance exercise was sufficient to cause

transient increases in the reaction of biochemical markers of bone formation and resorption due to a decrease in serum NTx (Whipple et al., 2004).

Predictors of BMD

Many studies have suggested that muscular strength is correlated with BMD and therefore has the ability to predict changes in regional bone density. Studies by Friedlander, Genant, Sadowsky, Byl, & Gluer (1995) and Lohman et al. (1995) support this theory with studies that showed large improvements in muscular strength associated with significant increases in the BMD of the lumbar spine in premenopausal women. Interestingly, Friedlander and colleagues implemented a protocol that combined aerobic and resistance exercises and found an increase in the femoral neck BMD, whereas Lohman et al. studied the effects of weight training over 18 months and found no significant increase in the femoral neck. The findings of Nickols-Richardson et al. (2007) suggested an increase in the total proximal femur and total body BMD in young adult women associated with increases in muscular strength. Unfortunately, this study did not assess changes in the lumbar spine. In contrast to these findings, studies by Vuori et al. (1994) and Heinonen et al. (1996) found significant increases in muscular strength in response to resistance training in young adult women without a corresponding increase in BMD.

Some critics of this association between muscular strength and bone density suggest that it is muscle mass and not muscular strength that is a more adequate predictor of BMD for the growing skeleton (Saggese & Baroncelli, 2002). Some investigators have found fat mass to be associated with BMD, but this correlation was only evident in

sedentary women (Madsen, Adams, & Van Loan, 1998). Other investigators have also found a positive relationship between total body weight and the BMD at the femoral neck in sedentary participants, but the correlation dropped to insignificance when athletes were included in the analysis (Reid, Plank, & Evans, 1992). When studying young women with different activity levels, Jurimae, Soot, & Jurimae (2005a) found that BMC was highly dependent on body mass in all groups while BMD was more dependent on LBM in endurance trained athletes, especially at the weight bearing sites of the lumbar spine and femoral neck. They also found lower-body anthropometric measurements to be the best predictor of BMC in weight-trained females. In a similar study, they found that leg BMD was dependent on muscle strength and anthropometric parameters; the dominant leg BMC and leg BMC were only dependent on LBM and isometric strength in non-athletic women; and the regime of strength measured (isokinetic or isometric) influenced BMC in sedentary groups (Jurimae, Soot, & Jurimae, 2005b).

Bell et al. (1985, p. 191) found that "obese people have altered calcium and vitamin D homeostasis, characterized by reduced ionized calcium activity, increased PTH levels, increased 1,25 (OH)2-vitamin D and reduced 25(OH)-vitamin D compared to non-obese people." Papakitsou et al. (2004) studied the effects of body mass index (BMI) on markers of bone formation and resorption in postmenopausal women. They found that PICP, a marker of collagen-I formation, was reduced in obesity with no significant changes between other markers of bone formation and resorption. Because the decreased concentration of PICP was not followed by a simultaneous decrease in BMD, they

concluded that obesity might have more of an impact on extraskeletal collegan-I than BMD (Papakitsou et al.).

There has been, however, an increasing amount of evidence supporting the relationship between lean body mass (LBM) and BMD. Sowers & Galuska (1993) found the greater the LBM, the greater the BMD in premenopausal women regardless of fat mass. Nichols, Sanborn, Bonnick, Gench, & DiMarco (1995) also observed that regional lean tissue mass was a better predictor of regional BMD than was regional fat mass. Madsen et al. (1998) found that there was not a significant correlation between LBM and BMD in sedentary subjects, but the correlation became significant when athletes were added into the analysis. When the athletes alone were analyzed, LBM was related to BMD at all sites including total body, lumbar and femoral neck BMDs (Madsen et al.). Therefore, the magnitude of the correlation between LBM and BMD is dependent on physical activity status. Thus, it can be concluded that LBM is a better predictor of BMD than is fat mass or muscular strength in physically active individuals.

Detraining

While in their original study, Vuori and colleagues (1994) found no significant gains in BMD following a resistance training regime in young adult women, they did find that the BMD decreased to baseline levels after 3 months of detraining (Vuori et al.). Kudlac, Nichols, Sanborn, and DiMarco (2004) found that after 4 years of retirement from competition, former female collegiate gymnasts continued to have a greater BMD at the proximal femur and total body than controls while both the gymnast and control groups had significant declines in BMD at the femoral neck, Ward's triangle, and greater

trochanter. Only gymnasts had a significant decline at the lumbar spine, suggesting a site-specific reaction to detraining (Kudlac et al.).

Based on the existing evidence, bone mineral density can be improved both by weight-bearing aerobic exercise and resistance exercise, with a combination of the two leading to the best results for total body BMD. While the effects of the volume of resistance training on markers of bone metabolism have not been thoroughly investigated, it is logical that an increased intensity of resistance exercise as well as an increased intensity and duration of aerobic exercise shows favorable effects on bone health. Finally, the beneficial effects of exercise on bone health are maximized only in the presence of adequate nutrient intake.

In summary, the evidence suggests that resistance exercise results in an intensity-related increase in markers of bone turnover. While an acute bout results in transient changes in markers, long duration studies with resistance exercise 6 days per week may result in lasting improvement to BMD. In addition, there is a site-specific effect of exercise on bone density. Therefore, improvement in bone density and markers of bone turnover may be greater and in long-term training interventions that implement exercises performed with a greater intensity and possibly volume.

Nutrition and Exercise Interactions

While a healthy diet plays an important role in bone health, nutrition and endocrine interventions alone cannot maintain or increase bone mass without the presence of weight-bearing activity (Murphy & Carroll, 2003). Calcium intake may influence the impact of physical activity in many people, especially children (Iuliano-Burns et al, 2003;

Specker & Binkley, 2003). The impact of Ca^{2+}, however, is more pronounced in those who are Ca^{2+} replete. In children, Ca^{2+} supplementation may increase bone mineral by 1-3% (Khan et al, 2001; Murphy & Carroll). However, the same effect of Ca^{2+} is not influential in adults. Devine et al. (1997) suggest that the effect of dietary Ca^{2+} may be less pronounced than genetic or other environmental factors in most premenopausal adults, but supplementation in the early postmenopausal period can have a positive effect on bone mass (Devine et al.). In this case, it would help maintain, but not increase bone mass in the long term (Khan et al.; McKane et al., 1996; Murphy & Carroll; Riggs et al., 1998). Short term changes may be seen after as little as 2 weeks of supplementation as evidenced by decreased bone resorption (Scopacasa et al., 1998). In addition Ginty et al. (1998) found similar changes in young adults. Further studies have found a larger effect size among subjects consuming greater than 1000 mg of Ca^{2+} per day (Kelley et al., 1998) and a reduced bone response to training among animals with low Ca^{2+} intake or bioavailability (Lanyon et al., 1986). A study by Specker and Binkley (2003) found that only children with high Ca^{2+} consumptions (>1100 mg/d) also displayed a positive effect of physical activity on leg bone mass. Additional studies have found that Ca^{2+} supplementation resulted in a general but not site-specific increase in bone mineral content (Murphy & Carroll, 2003).

CHAPTER III

METHODOLOGY

Participants and Design

This study used blood samples already collected from a study entitled "A comparison of the effects of an acute bout of low- and high-volume resistance exercise on insulin and glucose responses after an oral glucose challenge in premenopausal, normoglycemic women (Reed, 2008)." In that study, 10 women between the ages of 18 and 40 yrs completed two resistance exercise treatments and a resting treatment. The resistance exercise treatments included 1 (RE1) or 3 (RE3) sets of 10 resistance exercises. Each treatment was separated by 25-32 days to insure that each subject be tested during the follicular phase (4-11 days after the onset of menstruation) and to control for variations in the menstrual cycle. Fasting blood samples were collected 24 hr following each treatment.

Methodology for the Reed Study

One week prior to the Reed study, all participants underwent a VO_2max test determined by open-circuit spirometry during a graded treadmill exercise test protocol. The participants' body composition including total body tissue mass, fat mass, and bone mineral content was assessed by dual-energy X-ray absorptiometry (Lunar DPX-IQ) before and after completion of the study.

Blood Samples

Blood samples for the study were obtained between 8:30 –11:30 a.m. after a 12-14 hr overnight fast and 24 hr after each treatment. A 20 gauge x 1 in. catheter (Becton-Dickinson, Sandy, UT) was inserted into the antecubital vein and kept patent with 1 ml of a dilute sterile saline-heparin solution (10 U/ml). Prior to each sampling, 1 ml of venous blood was removed via the catheter and discarded. Samples (7 ml) were placed in blood collection tubes and centrifuged at 1800 x g for 8 min to obtain the serum. Serum samples were then stored at –70 °C for approximately 2 months prior to analysis.

Dietary Considerations

The participants were instructed to maintain a 3-day dietary record prior to each blood draw and consume a minimum of 150 g of carbohydrates per day. They were also instructed to consume a diet composed primarily of whole grains, vegetables, fruit, and lean protein. After the initial blood draw, the participants were supplied with a photocopy of their food record and instructed to repeat their diet prior to each of the following blood draws. In addition, each participant was provided the same meal (Subway sandwich, baked chips, and water) the night prior to each blood draw to avoid excess caloric consumption from fat. The meals consisted of 500-720 kilocalories with approximately 50% of the calories from carbohydrates, 20% protein, and 30% fat.

Resistance Exercise Protocol

Three bouts of resistance exercise were performed for the two resistance exercise treatments. The first bout consisted of a one-repetition maximum (1RM) on each piece of equipment using established procedures (Maud & Foster, 1995) and was performed on a Friday. The second bout took place the following Monday and consisted of one of the three treatments (Rest, RE1, RE3). Bout 2 was included to minimize muscle damage and soreness. Bout 3 consisted of the same treatment performed in Bout 2 and took place the following Friday (4 days after Bout 2) and 24 hr prior to blood sampling. The protocol for each treatment consisted of 10 repetitions performed at 65-70% of the participant's 1RM. Each repetition was performed at a 2 s concentric/2 s eccentric speed. This protocol was observed for both the 1 set and 3 set resistance exercise treatments (RE1 and RE3).

The resistance exercises were performed in a circuit in the following order: Cybex CV2 Dual-Axis Seated Row, Cybex CV2 Prone Leg Curl, Cybex CV2 Dual-Axis Seated Chest Press, Cybex CV2 Seated Leg Press, crunches, Cybex CV2 Dual-Axis Pulldown, Cybex CV2 Prone Leg Curl, Cybex CV2 Dual-Axis Overhead Press, Cybex CV2 Seated Leg Press, and Cybex 45° Back Raise machine. The machine exercises can be found in Appendix C. Upper body, lower body, and core exercises were alternated to minimize local muscular fatigue. Each set of resistance exercise in the circuit was initiated on a 2-min interval, and an additional 2 min of recovery was taken between the completion of each 10-set circuit and the initiation of the next 10-set circuit.

Analysis of Serum Samples

Serum samples obtained from the Reed study were analyzed for two markers of bone formation, osteocalcin and bone-specific alkaline phosphatase, and two markers of bone resorption, C-terminal collagen cross-links (CTx) and tartrate-resistant acid phosphatase (TRAP5b). All of the above markers are stable for up to 3 years while stored at –80 °C.

CTx was measured by a competitive-inhibition enzyme linked immunosorbant assay. The Serum CrossLaps ELISA is an enzyme immunological test used for the quantification of C-terminal telopeptides of Type-1 collagen breakdown products. The ELISA is based on two highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR, where the aspartic acid residue is β-isomerized.

Osteocalcin was measured by a Metra Osteocalcin competitive immunoassay that used osteocalcin coated strips, a mouse anti-osteocalcin antibody, an anti-mouse IgG-alkaline phosphatase conjugate, and a pNPP substrate to determine concentrations in serum. The assay was highly specific for intact OC and did not detect carboxy- or aminoterminal peptides. It also did not detect OC fragments released during osteoclastic resorption.

Bone-specific alkaline phosphatase (BAP) was measured using a Metra BAP immunoassay. It utilized a microtiter strip format using monoclonal anti-BAP antibody coating on the strip to capture BAP in the sample. A pNPP substrate was then used to detect the enzyme activity of the captured BAP.

Finally, TRAP5b was measured using a 2-step, Metra TRAP5b assay. It acts by using two different monoclonal antibodies, Trk49 and Trk62, which were generated with the immunization of purified TRAP5b from human bone cells. Trk49 binds inactive TRAP5b

fragments and leaves Trk62 more available to bind active TRAP5b in the microwell. Further explanation of the procedures can be found in the Appendix B.

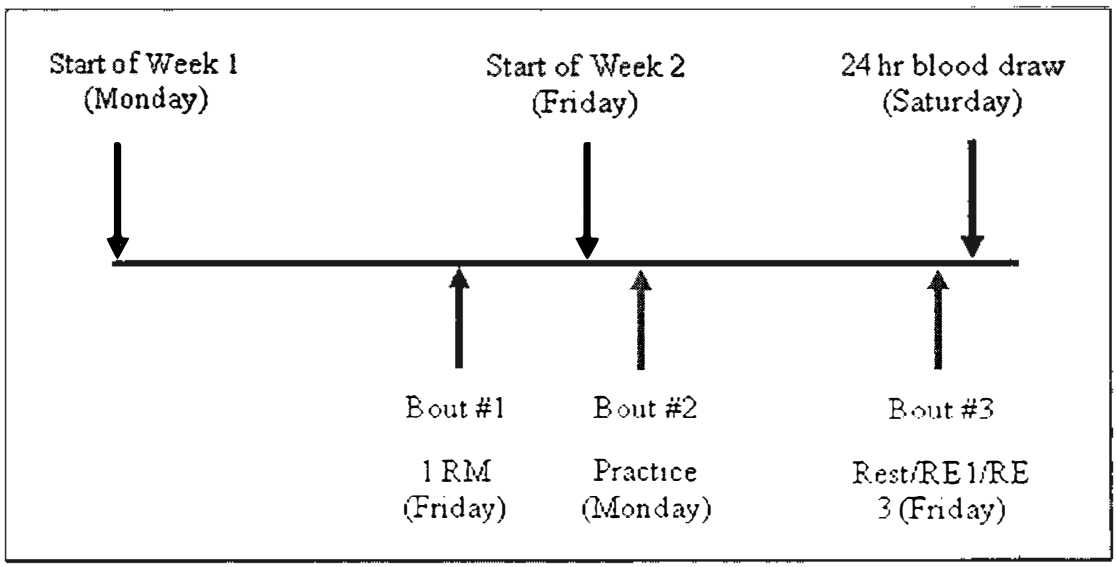

Figure 2. Body composition and the VO_2max of participants were determined at least 1 week prior to the start of the study. The 1RM was determined on Friday of Week 1. Bout 2 was performed 3 days later on Monday and consisted of one of the treatments (Rest, RE1, and RE3). Bout 3 repeated Bout 2 and was performed on Friday (96 hr after Bout 2), and the blood draw took place Saturday morning (24 hr after Bout 3). Weeks 1-2 of the study were repeated for Weeks 5-6 and 9-10 of the study.

Statistics

A one-way repeated measures MANOVA was used to compare changes in markers of bone metabolism following each treatment with the markers of bone metabolism being the dependent variables. A one-way repeated measures ANOVA was used to compare the ratio of bone formation to bone resorption following each treatment with the ratio being the dependent variable. Significance level was set at 0.025 to correct for multiple comparisons.

CHAPTER IV

RESULTS

The purpose of this study was to compare the effects of two resistance exercise treatments and a resting treatment on bone formation (OC and BAP) and bone resorption (CTx and TRAP5b) markers in 10 sedentary, premenopausal women. A one-way repeated measures MANOVA was used to compare changes in markers of bone metabolism following each treatment. A one-way repeated measures ANOVA was then used to compare the ratio of markers of bone formation to bone resorption following each treatment. The data analyzed in this study are discussed under the following headings: (a) Description of the Participants, and (b) Testing the Hypothesis.

Description of the Participants

A total of 10 premenopausal women (18-40 yrs) participated in the study. Four of the participants were < 30 yr of age while the other 6 were 30-40 yr of age. The ethnic diversity of the participants was 80% Caucasian and 20% African American.

Of the 15 participants that started the study, 10 participants completed all of the treatments. Three dropped out before the study began due to lack of interest. One participant dropped out after the 1st treatment, and a final participant was unable to complete the study after the 2nd treatment due to pregnancy.

Compliance to the resistance exercise protocol was 100% for all of the participants who completed the study. The resistance exercise was well tolerated by all participants, and no injuries were sustained as a result of the exercise.

Particpant characteristic and anthropometric measurements for baseline and post intervention are presented in Table 1. Changes in weight were not significant between baseline and post intervention. According to the World Health Organization classification chart on BMI and weight status, 4 of the 10 participants were of normal weight status, 2 were overweight, 1 was Obese class I, 2 were Obese class II, and 1 was Obese class III at baseline (WHO, 2000). One participant changed from Obese class II at baseline to Obese class III post-intervention. Mean macronutrient intake is presented in Table 3. All participants had a sufficient dietary intake of these nutrients.

Table 2.

Mean Baseline and Post Intervention Values for Anthropometric Measurements

Variable	Baseline	Post
Age	30.1 (9.1)	30.1 (9.1)
Weight (kg)	83.4 (25.8)	84.2 (25.9)
Height (cm)	167.8 (6.1)	-
BMI (kg/m^2)	29.5 (8.6)	29.8 (8.7)
Percent Fat Mass (%)	42.4 (12.2)	43.0 (11.3)
Percent Lean Tissue Mass (%)	57.6 (12.2)	57.0 (11.3)

Note. Values expressed as mean (± SD).

Table 3.

Mean Treatment Values for Macronutrient Intakes

Variable	Rest	RE1	RE3
Kcal	2215.2 (935.0)	2271.6 (483.7)	1758.9 (380.7)
FAT (%)	34 (7.2)	33 (7.1)	32 (4.9)
PRO (%)	16 (2.8)	14 (2.3)	16 (3.5)
CHO (%)	50 (5.8)	53 (7.4)	52 (7.3)

Note. Values expressed as mean (± SD).

Treatment values for bone formation and bone resorption markers are shown in Table 4. While not significant, OC increased from rest to RE3. The normal ranges for the markers can be found in Appendix B. Osteocalcin was slightly greater than the manufacturer's normal range while the TRAP5b results were slightly lower than the normal range for premenopausal women. Bone-specific alkaline phosphatase was also slightly higher than average with a large standard deviation.

Table 4.

Mean Treatment Values for Bone Formation and Bone Resorption Markers

	Bone Formation		Bone Resorption	
Variable	OC (ng/mL)	BAP (U/L)	CTx (ng/mL)	TRAP5b (U/L)
Rest	10.69 (2.20)	39.11 (16.56)	0.34 (0.63)	1.83 (0.69)
RE1	10.72 (1.62)	41.46 (26.81)	0.28 (0.18)	1.90 (0.71)
RE3	11.26 (2.05)	39.67 (17.57)	0.39 (0.33)	1.80 (0.72)

Note. Values expressed as mean (± SD).

Figures 3 and 4 below illustrate the changes in markers of bone formation to bone resorption. The results show that the ratio of OC to CTx increased slightly from rest to RE1, but was slightly decreased at RE3. On the other hand, the ratio of BAP to TRAP5b increased from rest to RE1 and was further elevated after RE3.

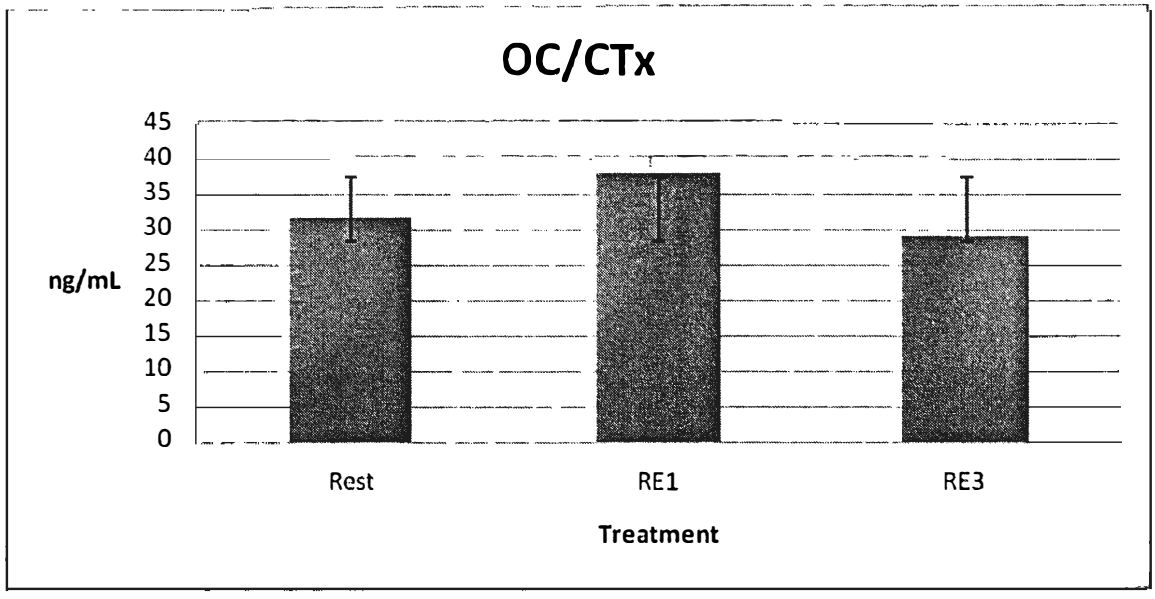

Figure 3. Ratio of OC to CTx

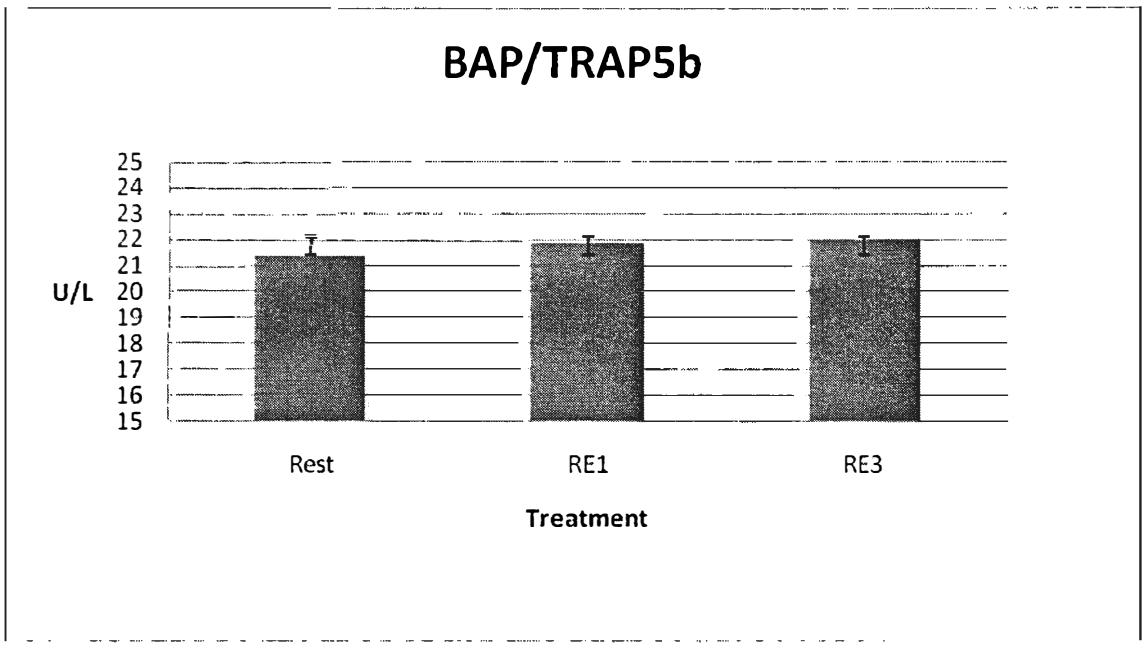

Figure 4. Ratio of BAP to TRAP5b

Testing the Hypothesis

A one-way repeated measures multivariate analysis of variance (MANOVA) was used to determine if any differences in markers of bone formation and resorption existed between treatments. A one-way repeated measures analysis of variance (ANOVA) was then used to determine if any differences existed between the ratios of bone formation to bone resorption markers between treatments. The findings were considered significant if $p < .025$. This significance level was set to correct for multiple comparisons.

The results of the one-way repeated measures MANOVA and the one-way repeated measures ANOVA are presented in Table 5 and 6. No significant differences were found

in either the markers of bone metabolism or in the ratio of bone formation to bone resorption between treatments.

Table 5.

Multivariate Analysis of Variance of Markers of Bone Formation and Bone Resorption

Value	F	Hypothesis df	Error df	P	Power
.264	.697	8.000	2.000	.706	.042

Note: Wilks' Lambda

Table 6.

One Way Repeated Measures Analysis of Variance of Markers of the Ratio of Markers of Bone Formation to Markers of Bone Resorption

Variable	SS	df	Mean Square	F	P
OC/CTx	69714.155	1.001	69616.024	.629	.448
BAP/TRAP5b	17.791	1.980	8.987	.830	.451

Note: Greenhouse-Geisser

CHAPTER V

DISCUSSION

The purpose of this study was to compare three treatments (rest, RE1, and RE3) and their effect on markers of bone formation (OC, BAP) and bone resorption (CTx, TRAP5b). The results of this study are discussed in this section under the following headings: (a) Summary, (b) Discussion, (c) Conclusion, (d) Recommendations for Further Studies.

Summary

A total of 10 sedentary, premenopausal women participated in the study. Their ages ranged from 18- 40 yrs, and the ethnic diversity consisted of 80% Caucasian and 20% African American.

Anthropometric measurements (weight and body composition) were taken at baseline and after the third treatment. During the intervention period, the participants were instructed to continue their regular activities and not increase physical activity or change their dietary consumption. Compliance to the resistance exercise intervention was 100%.

The dependent variables (OC, BAP, CTx, and TRAP5b) were analyzed by enzyme immunoassays. A one-way repeated measures multivariate analysis of variance was performed on all four dependent variables. A one-way repeated measures analysis of

variance was then run on the ratio of markers of bone formation to markers of bone formation.

After analysis of the data ($p < .025$), the following hypothesis were accepted:

1. There are no significant differences in markers of bone turnover between RE1, RE3, and rest in premenopausal women.
2. There is no significant difference in the ratio of bone formation to bone resorption markers in RE1, RE3, and rest in premenopausal women.

Discussion

The aim of this investigation was to study the effects of acute resistance exercise volume threshold on biochemical markers of bone metabolism in premenopausal women. Acute bouts of exercise with both low volume (1 set of 10 exercises) and high volume (3 sets of 10 exercises) resistance exercise showed no significant impact on biochemical markers of bone turnover compared to rest. While the results did not reach significance, OC and CTx did increase from the rest to the high volume treatment. In addition BAP and TRAP5b increased the greatest in the low volume treatment. Furthermore, neither resistance treatment resulted in a significant impact on the ratio of markers of bone formation to markers of bone resorption.

Strengths of the study, regarding the exercise intervention, included a high compliance rate. The resistance exercise compliance rate was 100% for the participants who completed the study most likely due to the short duration of the intervention and minimal time commitment required by the participants. In addition, all participants had an adequate intake of protein. Restricted energy or protein (or both) has a negative impact

on markers of bone metabolism while protein supplementation may result in increases of markers of bone formation (Ballard, Clapper, Specker, Binkley, & Vukovich, 2005). Because all of the participants in this study had an adequate amount of caloric consumption along with adequate amounts of protein, the results of this investigation should mostly be a reflection of the impact of the exercise treatment. However, there was a decrease in caloric intake for the high volume treatment. This could be considered a limitation since some markers may be variable based on caloric consumption (Bjarnason et al., 2002).

Another strength of the current study was that it controlled for the participant's menstrual cycle phase. Each treatment was timed to occur during the participants' follicular phase of the menstrual cycle (4-11 days after onset of menstruation) in order to control for variations between phases of the menstrual cycle. Studies have shown an increase in some markers of bone resorption during the follicular phase of the menstrual cycle. Glass, Kagam, Kohles, & Martens (2008) found significantly higher levels of serum CTx (9.46% increase from the luteal phase to the follicular phase) but no menstrual cycle variations were found in OC or BAP (Chiu, Ju, Mayes, Bacchetti, Weitz, & Arnaud, 1999; Glass et al.). Thus, studies that do not control for a specific phase of the menstrual cycle may have results that are influenced by fluctuations unrelated to the treatment.

Because of the fluctuation in markers due to the phase of the menstrual cycle, it is assumed that there was no difference in marker levels between cycles other than that influenced by the resistance exercise performed. One limitation of the study is that

multiple rest samples were not collected in order to compare within-subject variations from cycle to cycle.

Another limitation of the study was a lack of blood draw time points. In this study, blood samples were taken only at 24 hr post resistance exercise bout whereas other studies have found significant changes immediately post exercise as well as 1 and 8 hr following the treatment. A similar study in untrained men found a significant increase compared to controls in BAP immediately following a 45 min resistance exercise session consisting of three sets of seven exercises. However, it then returned to baseline at 1, 8, 24, and 48 hr postexercise (Whipple et al., 2004). In addition, serum NTx was significantly lower at 1 and 8 hr postexercise but not 24 hr postexercise compared to pretreatment values. There was an increase in the ratio of markers of bone formation to bone resorption (BAP: sNTx and PICP: sNTx) at 1 and 8 hr postexercise. Maimoun et al. (2005) found significant increases in BAP at 30 and 50 min during a 50 min steady state exercise test in 7 male cyclists both at 15% above and below the cyclist's ventilatory threshold. Osteocalcin was also significantly increased after 50 min of exercise performed at 15% above ventilatory threshold. Another study showed significant decreases in PICP and CTx 3 hr following a 60 min run in male athletes (Zitterman, et al., 2002). Finally, CTx was significantly elevated 30 min after the start of a 60 min 80% VO_2max cycle ergometer exercise in elite male triathletes and remained elevated 2 hr after the end of exercise. However, that study did not analyze blood samples 24 hr postexercise (Guillemant, Accarie, Peres, & Guillemant, 2004).

Because this study utilized samples already collected by the Reed study, we lacked the freedom to change the protocol to include additional time points. While we may have simply missed our window of opportunity to see significant changes in markers of bone formation and resorption, other studies have found significant results at 24 hr post exercise. One study found significant increases in total ALP in 9 young women 24 hr after brisk walking on a treadmill for 30 min carrying 5 kg of weight in a backpack (Tosun et al., 2006). Brahm et al. (1997) found an increase in OC after 24 hr of recovery following a maximal exercise test on the treadmill in 20 men and women. A study with 14 Asian male participants showed significant decreases in TRAP, urinary Dpd, and plasma PICP 24 hr following an acute bout of 3 sets of 10 repetitions of seven exercises. In addition serum BAP was significantly decreased 2 and 3 days after the exercise bout (Ashizawa et al., 1998). However, other studies that have found significant results 24 hr post exercise implemented an intervention with long duration training programs and not an acute bout of exercise (Ballard et al., 2005; Fujimara et al., 1997; Langberg et al., 2000; Mullins & Sinning, 2005; Shackelford et al., 2004; Vincent & Braith, 2002; Woitge et al., 1998).

In addition, the lack of dietary analysis for the intake of calcium and vitamin K was a limitation. Deficient amounts of calcium may limit the effects of an exercise intervention on bone metabolism (Kelley et al., 1998; Lanyon et al., 1986) while a surplus of calcium may reduce bone resorption with exercise (Guillemant et al., 2004). Vitamin K deficiency results in an increased percentage of ucOC that is associated with a decreased BMD and greater risk of fracture (Bugel, 2003; O'Connor et al., 2007). Previous studies showing

the effect of vitamin K supplementation on other markers of bone turnover found no significant results (Binkley et al., 2000; Bugel et al., 2007; Kruger et al., 2006) while Kalkwarf et al. (2004) found an inverse association between plasma phyloquinone and concentrations of NTx and osteocalcin. Because undercarboxylated OC serves as an indicator of vitamin K status, the lack of measurement in this study of undercarboxylated OC along with the lack of vitamin K intake data serve as two limitations of the study.

Also, sample size was another limitation of the study. Because the power in this study was low (.042 for between treatment comparisons of biomarkers, .448 for OC/CTx ratio, and .451 for BAP/TRAP5b), a greater sample size might be required for the results to reach significance. Only 10 women were used in the current study. While this is one more participant than Tosun et al. (2006) other studies included a greater number of participants (Ashizawa et al., 1998; Brahm et al., 1997).

Finally, one key limitation of the study is that there is no way of differentiating whether the changes in markers came solely from the final exercise bout performed for each treatment or if it was the result of a sum of the 3 bouts performed during the week. While there were 4 days between the practice bout on Monday and the treatment bout on Friday, it is unclear whether or not an adaptive effect to exercise may have influenced the marker concentrations. Participants in other studies measuring an acute bout did not perform any resistance exercise the week of the treatment (Ashizawa et al., 1998; Whipple et al., 2004). However, markers returned to baseline within 72 hours after completion of a single bout of exercise. Therefore, for the purpose of this study, an "acute

bout of resistance exercise" can only be defined by 3 different exercise bouts within one week with the bouts consisting of a 1RM for each exercise, a practice bout, and a treatment bout.

Conclusions

In conclusion an acute bout of resistance exercise does not result in significant changes in markers of bone formation and resorption or in the ratio of formation to resorption markers 24 hr postexercise in 10 premenopausal women. If serum was collected at multiple time points, the study included a greater sample size, or training was lengthened, a significant effect may have been seen, as evidenced by other studies.

Recommendations for Further Studies

1. A study where serum is collected before an acute resistance exercise bout as well as immediately post, 1 hr, 2 hr, 3 hr, 8 hr, 24 hr and 48 hr post intervention.
2. Studies that include a greater sample size.
3. Studies that establish an average rest value for each participant before implementing a treatment.
4. Studies that control for adequate intakes of macronutrients as well as calcium and vitamin K.

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APPENDIX A

Effect of Acute Exercise on Bone Markers

Table A1

Effect of Acute Exercise on Bone Markers

Study	Participants	Exercise	Duration	Timing of Blood Draw	Bone Resorption Markers	Bone Formation Markers
Brahm et al. (1997)	10 women 10 men	max treadmill test	10 min warm-up 10 min 47% VO_2max 10 min 76% VO_2max 4-5 min max till exhaustion	rest, immediately after each interval, after max exercise, 30 min post, 24 hr post	↑ PTH (during exercise and recovery)	↑ tALP (during exercise) ↑ PICP (during exercise)
Guillemant et al. (2004)	12 young male triathletes	cycle ergometer	60 min at 80% VO_2max	every 30 min: before exercise, during 60 min exercise, during 2 hr recovery	↑ CTx (30 min of exercise) ↑ CTx (2 hr recovery - 45-50%) ↑ PTH (30 and 60 min of exercise)	
Langberg et al. (2000)	7 male runners	marathon	26.2 miles	1 week before marathon, immediately after, 1,2,3,4,5,& 6 days after	↑ ICTP (immediately post)	↑ PICP (peaked at 72 hr)
Maimoun et al. (2005)	7 male cyclists	cycle ergometer	2 (50 min) steady state 1 at 15% +VT 1 at 15% -VT	Rest, 30 min of exercise, 50 min of exercise, after 15 min recovery	↑ CTx 11% for +VT (30 min) ↑ CTx 16% for +VT (50 min) ↑ PTH 41% +VT (50 min) ↑ PTH 80% + VT (15 min recovery)	↑ BAP 12% for -VT & +VT (30 min) ↑ BAP 12% -VT, 14% +VT (50 min) ↑ OC +VT (50 min)
Zitterman et al. (2002)	18 male athletes	treadmill	60 min at 70% max speed		↓ CTx approaching sig. (post)	↓ PICP (-9.8%)
Study	**Participants**	**Protocol**	**Resistance Exercises**	**Timing of Blood Draw**	**Bone Resorption Markers**	**Bone Formation Markers**
Ashizawa et al. (1998)	14 Oriental males	1 bout (3 sets of 10 reps) Set 1 - 60% 1RM Set 2&3 80% 1RM	7 exercises (bench press, back press, arm curl, leg extension, bent leg incline sit-up, lateral pull down, & leg press)	1600 and 0800 hr on control day, exercise day, & 3 post exercise days	↓ urinary Dpd Day 1 &3 post exercise ↓ TRAP (significant on Day 1 post exercise)	↓ PICP on exercise day and Day 1 post exercise ↓ BAP on Day 2 and 3 post exercise
Tosun et al. (2006)	9 women	treadmill	30 min brisk walking with and without 5 kg weight in a backpack	rest, 30th min of exercise, after 15 min recovery, & at 24 hr post	↑ PTH at 30th min with aerobic exercise with weights but returned to baseline after 15 min of recovery.	↓ tALP in aerobic exercise group 24 hr post ↑ tALP in weight-lifting group 24 hr post
Whipple et al. (2004)	9 men	1 bout (3 sets of 10 reps)	7 exercise (bench press, leg press, lateral pull down, arm curl, seated row, leg curl, & back extension)	immediately before, after, and at 1, 8, 24, & 48 hr post exercise	↓ NTx at 1, 8, & 48 hr post	↑ immediately post ↑ BAP:sNTx at 1 and 8 hr post ↑ PICP:sNTx at 1 and 8 hr post

APPENDIX B

Summary of Bone Marker Analysis Procedures

Summary of Bone Marker Analysis Procedures

BAP Enzyme Immunoassay Summary

1. Prepare the reagents and samples:
    a. Dilute 10x Wash buffer 1:10 with deionized water

2. Assay Procedure
    a. Pipette 125 µL of assay buffer into the assay wells.
    b. Pipette 20 µL of the standards, controls and specimens into the assay wells.
    c. Gently swirl the plate to ensure mixing of the samples with the buffer.
    d. Incubate for 3 hours ± 10 min at 20 – 28°C.
    e. While the plate is incubating, prepare the substrate solution 30-60 minutes before use.
    f. After the end of the incubation period, wash the plate 4 times with 1x wash buffer.

3. Pipette 150 µL substrate solution into each well.
4. Incubate 30 ± 5 min at 20-28°C.
5. Pipette 100 µL stop solution.
6. Read the plate at the optical density of 405 nm, and analyze the assay using a quadratic curve fit. ($y = A + Bx + Cx^2$).
    a. **Limits of detection**: The minimum detection limit of the Metra BAP assay is 0.7 U/L.

Table B1

BAP Reference Ranges

Gender	Age (years)	Range (U/L)	Median
Men	≥ 25	15.0 – 41.3	23.2
Women (Premenopausal)	25 – 44	11.6 – 29.6	25.0
Women (Postmenopausal)	≥ 45	14.2 – 41.3	23.2

Osteocalcin Enzyme Immunoassay Summary

1. Prepare the reagents and samples
    a. Dilute 10X wash buffer 1:10 with deionized water
    b. Within 2 hr of use, reconstitute each vial of enzyme conjugate with 10-mL of 1X was buffer.
    c. Within 1 hr of use, reconstitute standards and controls with 0.5 mL of 1X wash buffer. Freeze unused portions within 2 hr of reconstituting.
2. Assay Procedure
    a. Add 25 µL of the standards, controls, and samples in duplicate form to each well.
    b. Add 125 µL anti-Osteocalcin antibody to each well.
    c. Incubate 2 hours ± 10 min at 20-25 °C.
    d. Wash 3 times with 1X wash buffer using at least 300 µL of 1X wash buffer per well and blot the strips dry on paper towels after the last wash.
    e. Add 150 µL reconstituted Enzyme Conjugate.
    f. Incubate 60 ± 5 min at 20-25 °C.
    g. Wash 3 times with 1X wash buffer. Blot the strips dry on paper towels after the last wash.
    h. Add 150 µL Working Substrate Solution to each well.
    i. Incubate 35-40 min at 20-25 °C.
    j. Add 50 µL Stop Solution to each well to stop the reaction.
    k. Read the plate at the optical density of 405 nm, and analyze the assay using the equation: $y = (A-D)/(1+(x/C)^B)+D$.
        i. Limits of detection: Minimum analytical detection limit of the Metra Osteocalcin Assay is 0.45 ng/mL.
        ii. Observed range: 3.7 – 10.0 ng/mL (testing in laboratory of 140 adults)

TRAP5b Immunocapture Enzyme Assay

1. Prepare the reagents.
    a. Add 300 µL of deionized water to each vial containing lyophilized standard and control and dissolve for at least 5 min and mix thoroughly.
    b. Dilute 40 mL of 10X wash buffer with 360 mL deionized water.
    c. Prepare working substrate solution by adding 8 mL of substrate reconstitution buffer. This should be prepared within 30 min of use.
2. Assay Procedure
    a. Allow strips to equilibrate to 18-28 °C before opening.
    b. Pipette 100 µL sample diluent into the mircroplate wells.
    c. Pipette 50 µL of each reconstituted standard, control, and sample into duplicate microplate wells.

d. Seal the microwell plate and incubate for 60 min at 18-28 °C on a microplate shaker set at 500-1000 rpm.
e. Wash the microplate wells three times after incubation with a minimum of 300 µL of wash buffer per well. Tap the wells gently on a paper towel after washing.
f. Pipette 100 µL of working substrate solution into each well.
g. Seal the microplate and mix on a shaker for 30 seconds at 500-1000 rpm. Incubate for 60 min in a 37 °C incubator after shaking.
h. Pipette 50 µL of stop solution into each well to stop the reaction.
i. Read and record the absorbance of each well at 405 nm, and analyze the assay using a quadratic curve fit.
    i. **Limits of detection:** Minimum detection limit of Metra TRAP5b assay is 0.2 U/L.

Table B2

Observed Values for TRAP5b Activity in Healthy Men and Women

Gender	Age (years)	n	Mean (U/L)
Men	≥ 20	91	4.0 ± 1.4
Women (Premenopausal)	30 – 44	31	2.9 ± 1.4
Women (Postmenopausal)	≥ 50	36	4.3 ± 1.5

Serum CrossLaps ELISA (CTx)

1. Prepare the antibody solution no longer than 30 min before starting the assay. Prepare by mixing biotinylated antibody, peroxidase conjugated antibody, and incubation buffer in a 1+1+100 volumetric ratio in an empty container. Prepare a fresh solution before each run of the assay.
2. Pipette 50 µL standards, control, and samples in duplicate into each well.
3. Add 150 µL antibody solution to each well.
4. Incubate for 120± 5 min at 18-22°C on a mixing plate at 300 rpm.
5. Wash the immunostrips 5 times with washing buffer diluted 1+50 in distilled water.
6. Pipette 100 µL of the substrate solution into each well and incubate for 15± 2 min at room temperature in the dark on the mixing apparatus.

7. Pipette 100 µL of the stopping solution into each well.
8. Measure the absorbance at 450 nm with 650 nm as a reference within 2 hr.
    a. **Limits of detection**: The minimum detection limit of the Serum CrossLaps ELISA is 0.020 ng/mL.

Table B3

Observed Values for CTx Activity in Healthy Men and Women

Gender	n	Mean (ng/mL)	95% range
Men	125	0.294	0.115 – 0.748
Women (Premenopausal)	226	0.287	0.112 – 0.738
Women (Postmenopausal)	193	0.439	0.142 –1.351

APPENDIX C

Resistance Exercises

Cybex CV2 Dual-Axis Seated Row

Muscles:
Latissimus dorsi, Rhomboids,
Biceps brachii

Cybex CV2 Prone Leg Curl

Muscles:
Hamstrings

Cybex CV2 Dual-Axis Seated Chest Press

Muscles:
Pectoralis major, Anterior deltoids,
Triceps brachii

Cybex CV2 Seated Leg Press

Muscles:
Quadriceps, Hamstrings, Gluteals

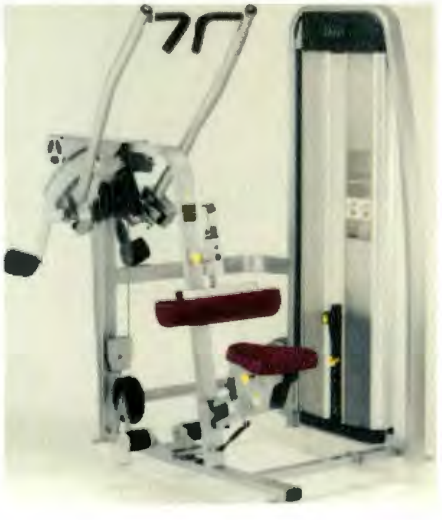
Cybex CV2 Dual-Axis Overhead Press

Muscles:
Deltoids, Triceps brachii

Cybex 45^{o} Back Extension

Muscles:
Erector spinae

Cybex CV2 Dual-Axis Pulldown



Muscles:
Latissimus dorsi, Biceps brachii