EXPLORING THE RELATIONSHIP BETWEEN CHANGES IN BONE MINERAL DENSITY, LEAN BODY MASS, AND HORMONES IN ACTIVE, ADULT MALES WITH OSTEOPENIA AFTER A 12-MONTH EXERCISE INTERVENTION

University of Missouri—Columbia

In Partial Fulfillment of the Requirements for the Degree Master of Science

by
MELISSA CARTER

Dr. Pamela Hinton, Advisor

DECEMBER 2012

The undersigned, appointed by the deal of the Graduate School, have examined the thesis entitled

EXPLORING THE RELATIONSHIP BETWEEN CHANGES IN BONE MINERAL DENSITY, LEAN BODY MASS, AND HORMONES IN ACTIVE, ADULT MALES WITH OSTEOPENIA AFTER A 12-MONTH EXERCISE INTERVENTION

presented by Melissa Carter,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Professor Pamela Hinton

_____

Professor John Thyfault

_____

Professor Mary Beth Brown

ACKNOWLEDGEMENTS

First of all, I would like to thank my Lord and Savior. "*I can do everything through Him who gives me strength."* Philippians 4:13.

My family is such an important part of my life, and their unfaltering love and support made this thesis possible. There are not enough words to describe my appreciation for my mother, Karen Carter. Without fault, she was always there for me. Her love, care, support, and patience did not go unnoticed. My brother, Jeff Rohr, my sister, Kristina Carter, and my step-father, Wayne Haselton, were a great source of stress relief during this manuscript. My family readily watched me practice my defense time and time again, they gave me encouragement when I needed it most, and they believed in me when I didn't believe in myself.

I would especially like to thank my advisor, Dr. Pamela Hinton, for her guidance and support throughout my graduate career and especially during completion of this research project. Her energy and enthusiasm for research is contagious, and I am very fortunate to have an advisor that is as invested in the success of her students as she. I thank her for guiding me toward success and for believing in me. I would especially like to thank her for her kind word at my thesis defense. This manuscript would not be possible with her guidance, support, and patience.

I would also like to thank my thesis committee members, Dr. John Thyfault and Dr. Marybeth Brown. They were insightful and their advice was of the upmost help. They were very patient with last minute revisions, and for that, I am forever grateful.

The other graduate research assistants, Andy Dawson, Tim Sinak, Jiang Jun, and the undergraduate research assistants, Lynn Eaton, Jaccalyn Billeter, Zach Wehmeyer, and Adam Younkin deserve a huge thank-you. The long hours, early mornings, and late nights put in by each and every person helped to make this study a success.

A huge thank-you goes out to Peggy Nigh, the study coordinator. She organized the lab, scheduled all the subjects, and she made sure everything was running as smoothly as possible.

There are many people that made this thesis possible, and without their contributions, this thesis would not be possible. Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

ABSTRACT

INTRODUCTION: Weight-bearing exercise may positively affect bone via muscle contractions, impact forces, and hormonal changes; however, the relative importance of these factors remains controversial. **PURPOSE:** Thus, we examined the effects of 12 months of resistance training (RT, a high-muscle-force activity) or plyometric (PLY, a high-impact activity) exercise on bone mineral density (BMD) in active (≥4hr/wk), osteopenic, but otherwise healthy men between the ages of 25 and 60y (mean: 42 ± 9.7y). We also examined the relationships between changes in lean body mass (LBM) and BMD of the whole body, weight-bearing (leg, hip) and non-weight-bearing (arm) sites. In addition, we examined the relationship between changes in BMD and changes in the concentrations of testosterone, free testosterone, and estradiol. **METHODS:** Participants were randomized to 12 months of supervised RT (2x/wk, N= 10) or PLY (3x/wk, N= 10). Each participant received supplemental calcium (1200 mg/d) and vitamin D (10 µg/d) for the duration of the study. LBM and BMD of the whole body, weight-bearing (legs and hips) and non-weight-bearing (arms) sites were measured at baseline and after the intervention using dual-energy X-ray absorptiometry. Testosterone, free testosterone, and estradiol concentrations were assessed using commercially available ELISA kits. Effects of RT or PLY on changes in whole body and regional (i.e., upper/lower body) LBM and BMD and hormone concentrations were evaluated using a 2x2 ANOVA (time, group). Relationships between percent changes in LBM or hormones and BMD were

assessed using Pearson's product moment correlations. **RESULTS:** Whole body (time, p= 0.025) and leg (time, p= 0.024) BMD significantly increased after the intervention with no differences between RT and PLY. By contrast, hip BMD increased in the RT group, but remained unchanged in PLY (group x time, p= 0.068). Whole body and leg LBM did not change significantly after 12 months of either RT or PLY. Arm LBM significantly decreased in the PLY group and remained unchanged in the RT group (group x time, p= 0.080). The percent change in whole body LBM was positively correlated with the percent change in left leg BMD (r= 0.581, p= 0.039) in the PLY group and was negatively correlated with the percent change in left leg BMD (r= -0.577, p= 0.040) in the RT group. The percent change in whole body LBM was positively correlated with the percent change in hip BMD (r= 0.565, p= 0.044) in the PLY group. The percent change in left arm LBM was positively correlated with the percent change in left arm BMD (r= 0.577, p= 0.047) in the RT group. There were no significant changes in total testosterone, free testosterone, or estradiol after the 12 month intervention in either group. The percent change in total testosterone was negatively correlated with the percent change in hip BMD in both the PLY (r= -0.643, p= 0.043) and the RT (r= -0.614, p= 0.039) groups. The percent change in estradiol was negatively correlated with the percent change in hip BMD (r= -0.735, p= 0.030) in the RT group. **DISCUSSION:** In conclusion, the results of the present study suggest that muscle contraction forces and impact forces may be potential mechanisms for osteogenesis in osteopenic men. Weight-bearing exercise that elicits either high- muscle-contraction forces or high-impact forces

may positively affect whole body and leg BMD, while hip BMD increased following high-muscle-contraction force exercise in physically active men with osteopenia.

Overall Objective

The overall objective of the proposed study is two-fold: 1) to determine the effects of 12 months of a plyometric or resistance training intervention on bone mineral density at weight-bearing and non-weight-bearing skeletal sites; and, 2) to explore potential mechanisms by which plyometric and resistance training exercise might increase bone mineral density in physically-active osteopenic men. The proposed mechanisms to be evaluated include lean body mass, gravitational loading, testosterone, and estradiol.

Specific Aims & Hypotheses

Specific Aim 1. To determine whether the relationships between changes in bone mineral density of weight-bearing sites (hips and legs) and changes in lean body mass of the lower body vary between a resistance training and plyometric intervention.

Hypothesis 1. We expect to see a positive relationship between the changes in lean body mass of the lower body and the change in bone mineral density of the hip and legs; we expect this relationship to be stronger in the resistance training group than the plyometric group.

Specific Aim 2. To determine whether the relationships between changes in bone mineral density and lean body mass of the non-weight-bearing sites (arms) vary between resistance training and plyometric exercises.

Hypothesis 2. We expect to see a positive relationship between the changes in lean body mass of the arm and changes in bone mineral density of the arm. This relationship will be the same in the resistance training and plyometric groups.

Specific Aim 3. To determine if changes in chronic testosterone, free testosterone, and estradiol concentrations will increase after 12-months of plyometric or resistance training interventions.

Hypothesis 3. We expect to see an increase in chronic testosterone, free testosterone, and estradiol concentrations in both the resistance training and plyometric groups.

Specific Aim 4. To determine whether the relationship between changes in testosterone, free testosterone, and estradiol concentrations and changes in bone mineral density of the hips, legs, and arms vary between the resistance training and plyometric interventions.

Hypothesis 4. We expect to see a positive relationship between the changes in testosterone, free testosterone, and estradiol concentrations and changes in bone mineral density of the hips, legs, and arms. In weight-bearing bones, we expect to see a stronger positive relationship between the change in the sex hormone concentrations and changes in bone mineral density of the hips and legs in the resistance training group. In non-weight-bearing bones, we expect both the resistance training and plyometric groups to have the same positive relationship between changes in the sex hormone concentrations and changes in bone mineral density of the arms.

Significance

Much of the research on prevention and treatment of osteoporosis has focused on women [1-3]; however, 35% of men have low bone mass and another 25% of men over the age of 50 y will endure an osteoporosis-related fracture [4-6]. The etiology of osteoporosis in men is multifactorial and may be related to declining gonadal function, an age-related decrease in osteoblast function, reduced mechanical loading, negative calcium balance, an increase in circulating parathyroid hormone, reduction in hormones (growth hormone, IGF-1, testosterone, estradiol), and/or a reduction in lean body mass [7]. Therefore, the significance of the proposed study was to identify effective exercise-based interventions to treat low bone mass in men and to better understand the relative contributions of lean body mass, gravitational loading, testosterone, and estradiol to increase bone mineral density in osteopenic men.

Current research has evaluated the effects of resistance training [8-10], plyometric exercises [2, 3], and the combined interventions [11, 12] on bone mineral density in women and young girls with promising results. The literature on physical-activity based interventions to increase bone mineral density in men is scarce. In the limited available research in men, Maddalozzo et al. [9] found that men had a greater osteogenic response to resistance training interventions that women [9, 11]. However, other research indicates that the response of bone mineral density to resistance training is not influenced by gender [10]. To date, the studies on the osteogenic effects of plyometric jumps on men are lacking. The purpose of the present study was to establish whether structured plyometric

activity and/or resistance training is as beneficial in men as it has been shown to be in women; as sex hormones, body weight, and body composition differ in regards to both genders.

The second purpose of the proposed study was to determine the relative contribution of changes in lean body mass and the high-intensity loading forces applied on bone to changes in bone mineral density at weight-bearing (hips and legs) and non-weight-bearing (arms) skeletal sites. In addition, we determined the effects of exercise-associated changes in testosterone and estradiol concentrations on bone mineral density of the hip, legs, and arms.

INTRODUCTION

Osteoporosis and its associated burden in men

Osteoporosis is a disease of the bone, in which bone tissue deteriorates and bone mass steadily decreases [13, 14]. The risk factors for osteoporosis include family history, age, low body weight, body composition, nutrition, smoking, excessive alcohol consumption, low calcium and/or vitamin D intake, low sex hormones, physical inactivity, and diseases or medications affecting bone metabolism [1, 6, 15]. The general loss in lean body mass and sex hormones as one ages is the cause of many age-related fractures, which affect millions of Americans [7, 13, 16-18].

Osteoporosis and bone-related problems are more prevalent in women than men [1], as post-menopausal estrogen deficiency is correlated with a rapid decline in bone mineral density [5, 6, 16, 18, 19]. Thus, most of the research on osteoporosis/osteopenia has focused on increasing and preserving bone mineral density in aging women [1, 10]. However, in the United States alone, more than 2 million men have osteoporosis, and another 12 million have osteopenia [1, 5, 6], which the World Health Organization defines as low bone density (T-score of -1.0 to -2.5, as compared to a T-score less than -2.5 for osteoporosis) [4]. Osteopenia is not a disease, but increases one's risk for osteoporosis in the future [4].

While rates of hip and vertebral fractures are higher in women than men, the rate of mortality-associated hip fractures are higher in elderly men [1, 20]. It

has been noted that 60-80% of all hip fractures and 70-90% of all spine fractures in men result from osteoporosis [1, 21]. In men, bone mineral density begins to decline as early as age 30 to 40, at a rate of 0.5-1.0% a year [22]. Due to the differences between the sexes in reproductive hormones, the rate of hormone released during and after exercise, body weight, fat and lean body mass [23], the mechanisms of bone loss in men are likely to be significantly different from those previously identified in women. Thus, research focused on bone disorders in men is desperately needed, as physical activity-based interventions in men may lead to a potential treatment of osteopenia.

Physical activity-based interventions

Resistance Training

Many studies that have examined the effects of a regular resistance training program on bone mineral density found promising results. Ryan et al. [10] studied the effects of a resistance training program in men and women with normal bone mineral density. After 6 months, they found an increase in femoral neck bone mineral density, but saw no change in the lumbar spine and whole body bone mineral density [10]. A similar study conducted on young men with normal bone metabolism saw no change in bone mineral density at any site measured after just 4 months of resistance training [24]. However, an average bone remodeling cycle can take up to 200 days [25]; thus, changes in bone mineral density may not be seen until the 6-7 month mark.

The type of resistance training used may also affect the outcome of the study. While weight machines are known to be a safer alternative than traditional free-weight resistance training, they do not load the skeleton to the same extent as free weights. Free-weight resistance training tends to be more functional, recruits stabilizing muscles, and it induces greater loads onto the skeleton [9]. Maddalozzo et al. [9] compared the effects of a 6-month high- or moderate-intensity weight training program on bone mineral density in both men and women. The moderate-intensity weight training program used weight machines and consisted of 10-13 repetitions at 40-60% of 1 repetition maximum (RM) [9]. The high-intensity weight-training program utilized free-weights at 70-90% of 1RM [9]. The high-intensity weight-training program induced an increase in bone mineral density at the spine (2%) and trochanter (1.24%) in the men and at the trochanter in the women (2.0%) [9]. Men in both the moderate- and high-intensity weight training programs improved bone mineral density of the trochanter (1.02% and 1.24%, respectively). While the machines and free weights differed in intensity, this study suggests that free weights induce a greater osteogenic stimulus than weight machines; however, more research is needed.

As previously mentioned, changes in bone mineral density as a result of resistance training may be different among men and women [26]. Almstedt et al. [26] had both male and female subjects in a periodized, free-weight, resistance training workout that was designed to load the hip and spine. The men experienced bone mineral density increases between 2.7-7.7%, while the women

exhibited changes ranging from -0.8-1.5% [26]. Thus, men seem to respond to osteogenic exercise differently than women. The control group lost an average of 1% bone mineral density; therefore, even though some women did not increase bone mineral density, the treatment may have suppressed the loss of additional bone. In many studies, the maintenance of bone mineral density is significant when compared with sedentary controls who lose bone as a natural consequence of aging [5, 13, 16, 27-30], and there is some evidence that resistance training can slow bone loss [10].

Plyometric Exercise

Plyometric jumps are a combination of eccentric and concentric muscle contractions that work via the stretch-shortening cycle [31]. Plyometric exercises induce a mechanical load that is dynamic, of short duration, and of greater magnitude than normally experienced [32]. Along with an increased strain rate, multi-planar movements and ground reaction forces that are many times greater than body weight, plyometric jumps may elicit an increase in bone strength and bone mass [32, 33]. Bailey et al. conducted a 6-month intervention study in which 21 premenopausal women did 50-multidirectional hops, on one leg, 2-7 times per week [2]. They found that bone mineral density at the femoral neck of the trained leg increased by 1.04% when compared to the control leg [2]. Another study compared walking with walking and jump training [3]. They found that the inclusion of jump training was effective in increasing or maintaining bone mineral density [3]. Conversely, a 12-week bilateral hopping intervention in

elderly men was ineffective in altering bone resorption and formation markers [34]. However, the same group conducted a similar study in young men, which indicated an acute response in bone turnover markers [34]. The lack of a response in the elderly men may have been due to a reduced mechanosensitivity, characteristic of aging bones [34].

The gravitational loading, or the impact force applied to bone, associated with a sport might significantly impact bone mineral density at weight-bearing sites (i.e. hips and legs). Athletes involved in high-impact or high-power sports tend to have greater bone mineral density and lean body mass than those in endurance sports [13, 35]. In high-impact activities, the muscle pulls on the bone, causing it to momentarily bend [27-29, 36, 37]. The resulting strain on the skeleton is a stimulus for new bone accretion [27-29, 36, 37]. In non-weight bearing activities, such as cycling or swimming, the resulting pull of the muscle's contraction is similar to that in other endurance sports, such as running. An important difference between a weight-bearing endurance sport (i.e. running) and a non-weight-bearing endurance sport (i.e. cycling) is the gravitational loading of the bones. Thus, even with the contraction of large muscle groups, the lack of the gravitational loading of body weight may decrease the strength and force output of the muscular contractions in weight-bearing sites (i.e. legs). Thus, with a decreased level of strain on the skeleton in weight-bearing sites, the stimulus to initiate bone growth is not as strong as in weight-bearing activities, leading to decreased turnover of bone in sites such as the hips and legs.

Activities, such as plyometric jumps and gymnastics, which induce high-strain rates distributed unevenly across the bone, provide the greatest osteogenic stimulus [28, 35]. Creighton et al. [13] compared the bone mineral density of athletes in high-impact (i.e. basketball, volleyball), medium-impact (i.e. soccer, track), non-impact sports (swimming), and sedentary controls. They found that those in high-impact sports had the highest bone mineral density at weight-bearing sites, and they had greatest markers of bone formation [13]. They also found that the bone mineral density at weight-bearing sites did not differ between the non-impact sports and sedentary controls [13]. In agreement, Duncan et al. [14] found that triathletes and runners had significantly higher bone mineral density of weight-bearing sites than cyclists, swimmers, or sedentary controls. It has been suggested that the application of fast, powerful muscular contractions to the bone may strengthen the bone formation process [35].

Several cross-sectional studies have compared the effects of weight-bearing and non-weight-bearing sports to determine the effect of strength, lean body mass, and ground reaction forces on the osteogenic response in bone [6, 14]. It is currently accepted that bone-loading and ground reaction forces may elicit an osteogenic response among osteopenic females [14]; however, to our knowledge, no study has examined the change in bone mineral density with respect to a change in body composition over time in osteopenic men.

Animal-based research

Several studies have evaluated the osteogenic response of exercise-based interventions in rats [33, 38-40]. As few as 10 jumps per day increased the bone formation rate in rats when compared to controls; however, 40 and 100 jumps increased that response even further [33]. The bone formation rate was not significantly different between the 40 to the 100 jump groups [33]. This finding parallels an earlier study by the same research lab, which indicated that only 5 jumps per day was effective at increasing bone mineral density in rats [40].

Interestingly, it has been suggested that the plyometric-induced osteogenic gains in rats are preserved even after a 24-week detraining period. Bone mineral density decreased over the course of the 24-weeks, but the percent change from baseline was still positive [38].

Physical activity-induced increases in bone mineral density

This study will examine three possible mechanisms by which physical activity increases bone mineral density: 1) lean body mass, 2) gravitational loading, and/or 3) changes in the sex hormones testosterone and/or estradiol.

Muscle Contractions

Bone cells are sensitive to the mechanical forces exerted by the muscle [41]. During a muscle contraction in high-impact activities, the muscle pulls at the attachment site on the bone, and it causes the bone to momentarily bend as it reacts to the pulling forces [27-29, 36, 37]. The greater forces (i.e. body weight and muscle mass) exhibited on the bone, the greater the strain on the skeleton.

The resulting strain on the skeleton is a stimulus for new bone accretion [27-29, 36, 37]. To be osteogenic, a muscle contraction must be dynamic, of sufficient magnitude, and imposed at significant rates [41]. Thus, with increases in lean body mass and strength, the forces exerted by the contracting muscle will be greater, thereby, inducing a greater strain on the bone, which increases new bone accretion.

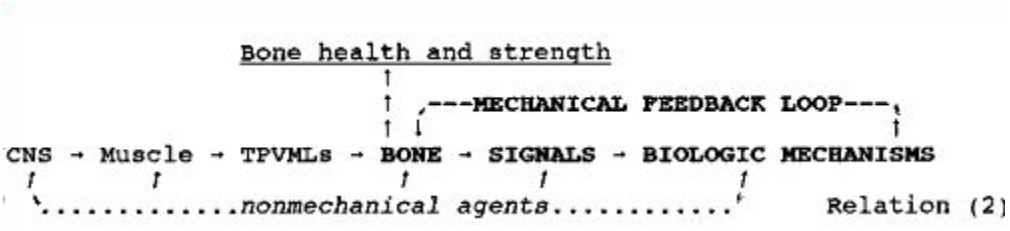


FIGURE 1. Diagram of the potential way in which muscle contractions can cause increases in bone mineral density. CNS: central nervous system; TPVML: typical peak voluntary mechanical load. Adapted from Frost, 2003 [42].

Lean muscle mass and strength

Several studies have found evidence for a relationship between muscular strength and bone mineral density [14]. It has been suggested that the positive relationship between strength and bone mineral density may be due to an increase in lean body mass [14]. Some data suggest that an increase in lean body mass, independent of muscular strength, may increase gravitational loading and compressive forces [14], which may generate strain on the bone, and induce a change in bone mineral density [14]. Research on men is needed in this area,

as it has been suggested that the lean body mass, strength, and bone mineral density relationship may differ between the sexes [14].

Gravitational Loading

Weight-bearing and non-weight-bearing physical activity

Physical inactivity is a modifiable risk factor for osteoporosis; however, to obtain the skeletal benefits of physical activity in terms of gravitational loading, at least a small proportion of the activity must be weight-bearing in nature [5, 13, 28]. Several studies have shown that including a modest amount of weight-bearing activity in one's workout regime is necessary to gain the full benefits in terms of bone health [13, 28]. It has been suggested that men at risk for osteoporosis should participate in 30-40 minutes of weight-bearing activity at least 3 times a week [22].

Weight-bearing activity is an important part of skeletal health due to the osteogenic effects that ground reaction forces may exert on the skeleton. Even though an increase in lean body mass may contribute to stronger muscle contractions, and therefore, a potential driving stimulus for bone accretion, the forces that the ground exerts on the body may increase bone density as well. Ground reaction forces are used to quantify the degree of loading on the skeleton during weight-bearing exercise by using multiples of body weight [43]. For example, walking has a ground reaction force of 1.2, while running would have a ground reaction force around 2.6, and a depth jump would have a ground reaction force of 5.2 [43]. Ground reaction forces are a reliable measure of bone

strain, and are frequently used to report exercise intensity as it relates to skeletal loading [43].

In activities such as distance running and high-impact sports, it has been proposed that the ground reaction forces stimulate bone turnover, and therefore increase reabsorption and formation markers in blood [5]. The mechanical stress associated with gravitational loading is not seen in non-weight-bearing sports, such as cycling and swimming. Nichols et al. [28] found that compared to age-matched sedentary controls, male elite cyclists had a 10% lower bone mineral density at the femur and spine. Other studies have found cyclists to have lower bone mineral density than weight-bearing aerobic sports, such as long-distance running [5, 14, 44, 45]. It has been postulated that muscular force is the driving stimulus for bone mass accretion [13, 27, 28]. However, since cyclists and runners experience similar and significant muscle contractile forces, and cyclists consistently have lower bone mineral density than runners, bone loading via ground reaction forces seems to be an important predictor of bone mineral density.

Thus, weight-bearing activities have been associated with an increased bone mineral density, and may increase bone mineral density when the stimulus is great enough [13, 14, 29, 46]. Studies that have compared adult athletes in weight-bearing sports and non-weight-bearing sports have shown a positive relationship between weight-bearing exercise and skeletal health [14, 28, 29, 35-37]. Creighton et al. [13] found that young women who participated in high-impact sports (i.e. basketball and volleyball) displayed a greater bone mineral

density at weight-bearing sites when compared to medium-impact sports (i.e. soccer and track), non-impact sports (i.e. swimmers), and controls. Thus, the amount of impact exerted on the skeleton on a regular basis may elicit greater increases in bone density.

Forces on the upper limb

Due to the close association between gravitational loading and muscle contractions, it is difficult to separate the independent effects of each bone-driving stimulus. While it may be near impossible to independently measure the effects of gravitational loading, isolation of muscle contractile forces can be achieved using the upper limb. The arm bones are exposed to muscle contraction forces, but are not subjected to ground reaction forces as they do not transmit body weight to the ground [41]. A review article that examined the relationship between muscular loading and bone mass described several studies that associated changes in bone mineral density with changes in lean body mass, in both the upper arm and whole body [41]. This review, by Robling [41], demonstrated that muscle forces provide a large amount of strain and force on the bone; furthermore, muscle forces may be sufficient enough to drive bone adaptions.

To further support the importance of muscle contractile forces, Umemura et al. [47] designed an experiment in which rats were trained to jump up to an elevated platform (0.5 meters). The rats caught the edge of the platform with their front paws and used their upper body to climb up to the top of the platform

[47]. This model uses explosive muscle contractions, while separating out the impact of ground reaction forces. After jumping 100 times per day, 5 days a week, for 8 weeks, both young and old rats had a significant increase in bone mass [47]. The increase in bone mass in the jump-trained rats was significantly greater than in run-trained rats, and the increase in bone mass in the run-trained rats was significantly greater than in sedentary rats [47]. Thus, activity that induces strong muscle contraction forces may be a means to positively influence bone.

Testosterone

Influence of testosterone on bone mineral density

Testosterone is an androgenic-anabolic hormone, and its biological effects include stimulating muscle hypertrophy through increased protein synthesis and the inhibition of protein degradation [48]. The average adult male produces 7mg of testosterone daily [49, 50], and there is a gradual decline in testosterone production with aging [7, 20, 32]. As testosterone production decreases, there is a related decline in muscle and bone mass, and an associated increase in fat mass [7, 32]. The administration of testosterone to aging men can be beneficial for bone mineral density and body composition [7].

Testosterone has been identified as a driving stimulus for lean body mass and strength gains, but it has also been associated with positive effects on bone mineral density. Testosterone therapy has been suggested as a viable treatment for men at high risk of fracture who have testosterone levels less than 6.9

nmol/liter, such that normalization of testosterone levels has been related to increases in bone mineral density [22]. Isidori et al. [32] conducted a meta-analysis on the effects of testosterone treatment on body composition and bone metabolism in middle-aged men. The studies averaged a decrease of 6.2% body fat and an increase of 2.7% lean body mass with testosterone treatment [32]. Nearly all of the analyzed studies showed that the testosterone group had greater bone mineral density of the lumbar spine and femoral neck (+3% and +2%, respectively) than the control group [32]. It is unclear whether testosterone had an indirect or direct effect on the bone. Indirectly, testosterone may have induced an increase in lean body mass. The increase in lean body mass contributes to increases in muscle contractile forces, thus inducing greater strain rates on the bone. On the other hand, testosterone may directly target the bone inducing bone turnover [51]. With that being said, an increase in testosterone has been associated with an increase in lean body mass and bone mineral density in middle-aged men with low testosterone levels [32].

Effects of resistance training on testosterone

Resistance exercise increases the concentration of many hormones, including testosterone [52]. The increase in hormones may be due to one of four factors: 1) an increase in the secretion of the hormone, 2) a decrease in hepatic clearance, 3) plasma volume reduction, or 4) reduced degradation states [52]. Regardless of the method of increase in hormone concentrations, the increase in hormones presents a greater likelihood of interaction with receptors in target

tissues [52]. Greater testosterone responses are experienced in workouts that include large muscles mass exercises (such as the clean and jerk, snatch, deadlift, and jump squats), high volume sessions at a moderate to high intensity, with short rest intervals [52-54].

The myofibrillar disruption in response to a resistance training workout induces an inflammatory response [53]. An acute resistance training workout elevates concentrations of testosterone, growth hormone, cortisol, and certain cytokines [53]. Cytokines are small, signaling, protein molecules that can be immunological or inflammatory in nature. An increase in testosterone may suppress pro-inflammatory cytokines (tumor necrosis factor-α, interleukin-1β, and interleukin-6) and potentiate the expression of anti-inflammatory cytokines (interleukin-10) [53, 55]. Current research suggests that inflammation may contribute to bone loss [56] as high concentrations of inflammatory markers are associated with an increase in fracture risk [56, 57]. Thus, if resistance training increases testosterone concentrations, the increase in testosterone may suppress inflammatory cytokines, therefore reducing bone loss.

The hormonal response to exercise is muted as the body adapts to the stress. After a 7-week periodized resistance training program, Izquierdo et al. [53] measured testosterone concentrations in men at either the same relative intensity or absolute intensity in relation to pre-training testing. Serum total testosterone and free testosterone concentrations increased during and after the resistance training period, but returned to pre-exercise values within 45 minutes post-exercise [53]. Mid-exercise and post-exercise free testosterone

concentrations were greater with the relative load than the same absolute load [53]. Thus, workouts that employ the same relative load tend to induce greater muscle hypertrophy and are needed to maintain a training stimulus in order to keep seeing changes muscularly and hormonally.

Effect of plyometric exercises on testosterone concentrations

It is well known that resistance training induces increases in testosterone concentrations [31, 32, 52-54, 58-62], but the effects of plyometric exercises on testosterone concentrations are less clear [31, 32, 62]. However, due to the intense nature and eccentric component associated with plyometric activity, much of the current research points to an increase in testosterone concentrations following jumping exercise [31, 32, 62]. After 60 seconds of continuous jumping exercise in professional soccer players, total testosterone and free testosterone were both significantly increased from pre-jumping values [62]. Therefore, these results may suggest that the inclusion of explosive-type activities may be associated with an increase in testosterone concentrations [62].

Plyometric exercise has also shown to elicit an immediate [62] and prolonged [31] increase in testosterone concentrations from previous levels. In an opposing plyometric-exercise study, testosterone concentrations did not increase immediately post-exercise; however, they did significantly increase above resting values 48 to 72 hours later [31]. Chatziwikolaou et al. [31, 52] suggested that this delayed increase in testosterone may be a homeostatic response to offset the post-exercise catabolic state.

Direct effect of testosterone on bone turnover

While the current literature suggests that sex hormones influence bone, the method by which bone adaptions take place is less well known. It is unclear whether the sex hormones directly or indirectly cause bone adaptations, and it very well could be that testosterone exerts both direct and indirect skeletal adaptions. The indirect effects of testosterone may be mediated via increased muscle mass; thus, the stronger muscle contractile forces initiate adaption in the bone such that the bone can resist the more intense forces. On the other hand, the current research has found bone cells to have both testosterone and estrogen receptors [63]. It has been suggested that testosterone may directly inhibit osteoclast formation and bone resorption [63], thereby reducing the breakdown of bone. While testosterone may influence osteoclast formation and bone resorption, it has been suggested that the effect of estrogen on osteoclasts is mediated by osteoblasts [63].

Estrogen

Influence of estrogen on bone mineral density

Due to the dramatic increase in osteoporosis after menopause in women, it is well-known that estrogens are an important factor regarding bone health [64]. It has been suggested that estrogen deficiency may be responsible not only for the rapid reduction in post-menopausal bone loss, but also for the slow age-related decline in bone mass seen in both men and women [65]. With that being said, the current literature recognizes estrogens as potentially more important

than androgens for the maintenance of skeletal health, normal bone formation, and suppression of bone resorption in men [66]. In a cross-sectional study, bioavailable estradiol was the most consistent predictor of bone mineral density in men [65]. Ackerman et al. [66] found that total and free estrogens are stronger predictors of bone mineral density than testosterone, and may be a significant determinant of bone mineral density in male athletes.

In both men and women, bioavailable estrogen is the sex steroid with the strongest association with bone mineral density [67]. In men, total estrogen levels remain relatively stable throughout the lifespan, such that older men tend to have estrogen levels that are at least twice that of post-menopausal women [67]; however, due to binding with sex hormone binding globulin (SHBG), the bioavailable form of estrogen decreases with advancing age [65, 68]. Thus, skeletal health suffers due to the decline in the bioavailable form of estrogen. Therefore, total and bioavailable estrogen levels decrease in women due to a decrease in ovarian estrogen production, and they decrease in men due to an age-related increase in SHBG.

As mentioned, bioavailable estrogen concentrations are positively correlated with bone mineral density, and they are a consistent predictor of bone mineral density in aging men [65]. The current literature suggests that estrogen is the best predictor of the attainment of peak bone mass in young men and the decrease of bone mass in elderly men [65, 69, 70]. In healthy elderly men, serum total estrogen was found to have a strong positive association with bone mineral density at all sites, and free estrogen was found to have a slightly

stronger relationship with bone mineral density when compared to total estrogen levels [67]. Araujo et al. [71] found that while neither total nor free testosterone were related to bone mineral density, both total and free estradiol were related to bone mineral density. Thus, this suggests that estrogens, as opposed to androgens, are the best predictor of bone health and maintenance in men.

Sex Hormone Binding Globulin

Sex hormone binding globulin (SHBG) is a glycoprotein that binds to sex steroid hormones, i.e. testosterone and estradiol, thereby inhibiting the function of these hormones [58]. The sex steroids testosterone and estradiol can be bound to specific or unspecific proteins, remain unbound (free), or give rise to conjugate steroids [58]. Thus, the bioavailability of these hormones is limited by the proportions that are bound to SHBG. Due to its affinity to bind to testosterone and estradiol, thereby rendering them inactive, SHBG has an inverse relationship with bone mineral density [20].

Most testosterone and estradiol circulate in the blood stream either bound to SHBG, albumin; however, a small percentage of total testosterone and estradiol remains biologically active, i.e. free (free testosterone and free estradiol) [58]. These free sex hormones are able to enter a cell and activate its receptors, while bound testosterone and/or estradiol is inactive [52]. As one ages, the concentration of SHBG increases [20, 72]. Thus, the amount of free testosterone and free estradiol declines, while total testosterone and total estradiol remains unchanged [7, 72]. With that being said, low concentrations of

free testosterone/estradiol and high concentrations of SHBG are independently and additively associated with an increased risk of fracture in men [57].

Hypothetical framework

Therefore, at weight-bearing skeletal sites, both resistance training and plyometric jumping may be osteogenic due to the effects of sex hormones and muscle contractions, but only plyometric exercise exerts increased gravitational loading (**Figure 2**). At non-weight-bearing skeletal sites, i.e. arms, resistance training and plyometric jumps may be osteogenic due to the effects of testosterone, estradiol, and muscle contractions (**Figure 3**). The focus of the present study was to explore muscle contractions, gravitational loading, and hormonal changes as potential mechanisms through which bone mineral density may increase after 12-months of resistance training or plyometric exercise in physically-active osteopenic men.

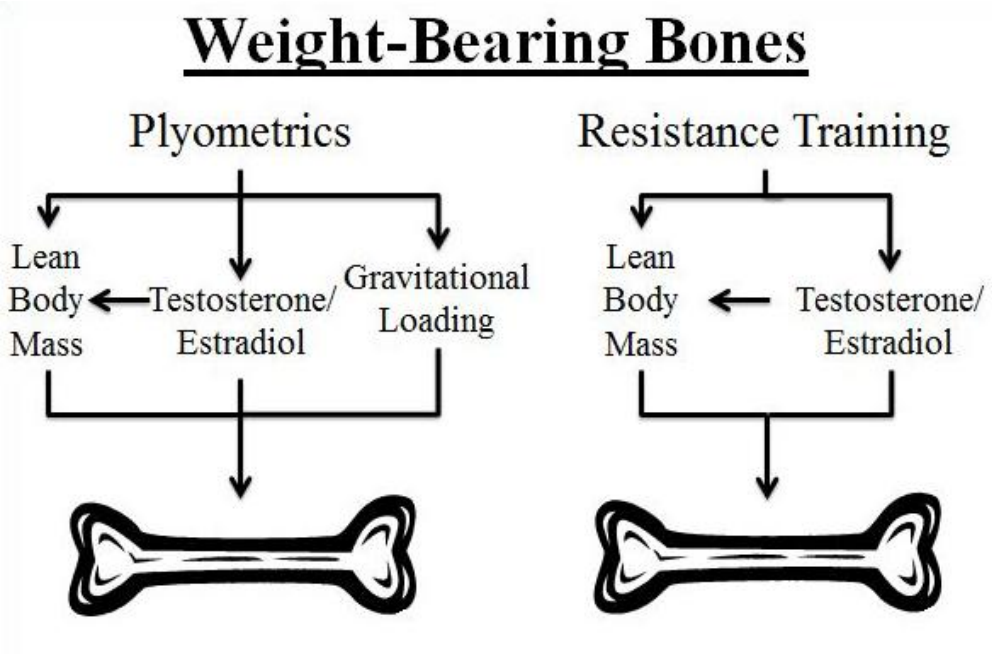


FIGURE 2. The proposed effects of plyometric and resistance training exercise on increasing bone mineral density at weight-bearing skeletal sites. The added effect of gravitational loading during plyometric exercise should weaken the association between lean body mass and bone mineral density. Furthermore, resistance training should see the greater association between lean body mass and bone mineral density.

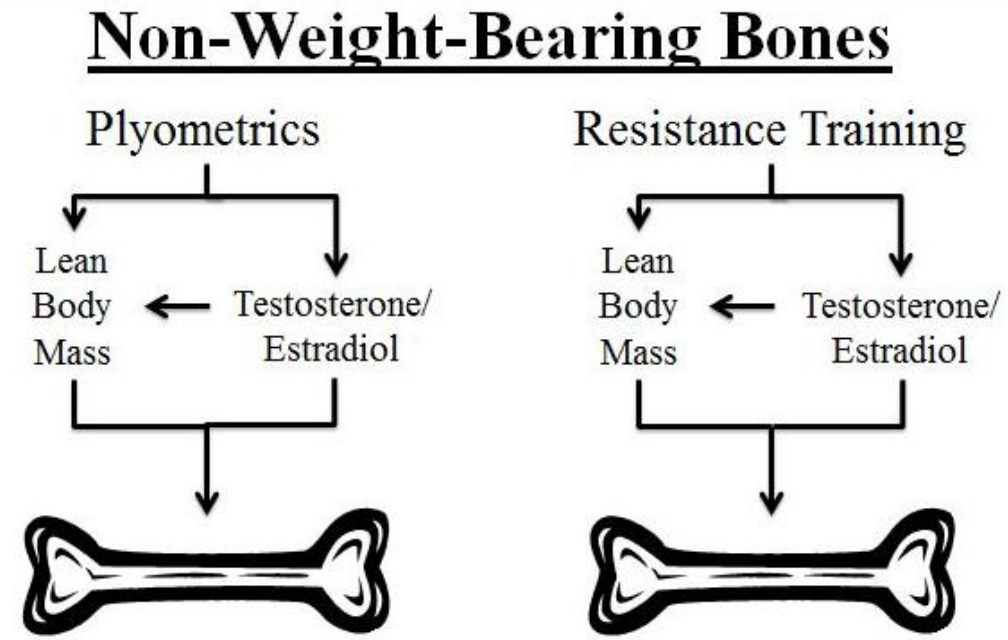


FIGURE 3. The proposed effects of plyometric and resistance training exercise on increasing bone mineral density at non-weight-bearing skeletal sites. Since both interventions have the same factors contributing to an increase in bone mineral density, the relationship between lean body mass and bone mineral density should be the same in both intervention groups.

Purpose

The overall purpose of the present study was two-fold: to determine the effects of a 12-month plyometric or resistance training intervention on bone mineral density at weight-bearing and non-weight-bearing skeletal sites; and to explore potential mechanisms by which plyometric and resistance training exercise might increase bone mineral density in physically-active osteopenic men. The mechanisms to be evaluated included lean body mass, gravitational loading, and changes in sex hormones.

Specific Aim 1. To determine whether the relationships between changes in bone mineral density of weight-bearing sites (hips and legs) and changes in lean body mass of the lower body vary between the resistance training and plyometric groups.

Hypothesis 1. We expected to see a positive relationship between the change in lean body mass of the lower body and the change in bone mineral density of the hip and legs, and we expected this relationship to be stronger in the resistance training group than the plyometric group.

Expected outcomes 1: Refer to **Figure 2** and **Figure 4.**

When examining the correlation between the percent change in bone mineral density of the hip and leg and the percent change in lean body mass of the lower body after a 12-month intervention, we expected to see a positive relationship in both groups, but a stronger relationship in the resistance training group when compared to the plyometric group. In plyometric exercise, three factors contribute to an increase in bone mineral density: an increase in muscle contractile forces, an increase in gravitational loading, and an increase in hormones. In resistance training, an increase in bone mineral density is due to an increase in muscle contractile forces and an increase in hormones. Since gravitational loading influences bone mineral density in the plyometric group, we expected the relationship between percent changes in hip and leg bone mineral density and percent changes in lower-body lean body mass to not be as strong in the plyometric group as in the resistance training group.

Specific Aim 2. To determine whether the relationships between percent changes in bone mineral density and lean body mass of the non-weight-bearing sites vary between the resistance training and plyometric groups.

Hypothesis 2. We expected to see a positive relationship between the percent changes in lean body mass of the arm and percent changes in bone mineral density of the arm. We expected this relationship to be the same in the resistance training and plyometric groups.

Expected outcomes 2: Refer to **Figure 3** and **Figure 5.**

Due to the arms being a non-weight bearing site, the ground reaction forces associated with plyometric jumps will not have an effect on the change in bone mineral density or the change in lean body mass. Thus, both intervention groups depended on muscle contractions in the arms to stimulate bone growth.

Therefore, since the bone mineral density of the arms is purely dependent on lean body mass and muscle contractile forces, we expected the relationship between the percent change in bone mineral density of the arms and the percent change in lean body mass of the arms to be the same in the resistance training and plyometric groups.

Specific Aim 3. . To determine if changes in chronic testosterone, free testosterone, and estradiol concentrations will increase after the 12-month plyometric or resistance training intervention.

Hypothesis 3. We expected to see an increase in chronic testosterone, free testosterone, and estradiol concentrations in both the resistance training and plyometric groups.

We chose to measure free as well as total testosterone concentrations because the free portion of testosterone is the bioavailable portion. Free testosterone is not bound to proteins and is available for activation.

Current research has shown testosterone to increase following either resistance training or plyometric exercises, and while there is a lack of research on the effects of estradiol and plyometric jumps, estradiol has been shown to increase after resistance training [31, 32, 52-54, 58-62, 66]. Both resistance training and plyometric exercises have eccentric components, and eccentric exercise is known to induce the greatest strain and damage to the muscle [31]. Thus, in response to the myofibrillar damage, testosterone concentrations will increase. Concentrations of estradiol have been reported to increase after resistance training exercise, and may be due to the increased conversion of testosterone to estrogen [73-75].

Specific Aim 4. To determine whether the relationship between changes in testosterone, free testosterone, and estradiol concentrations and changes in bone mineral density of the hip, legs, and arms vary between resistance training and plyometric groups.

Hypothesis 4. We expected to see a positive relationship between the percent changes in testosterone, free testosterone, and estradiol concentrations and the

percent changes in bone mineral density of the hips, legs, and arms. In weight-bearing-bones, we expected to see a stronger positive relationship between the percent changes in the sex hormone concentrations and the percent changes in bone mineral density of the hips and legs in the resistance training group than in the plyometric group. In non-weight-bearing bones, we expected both the resistance training and plyometric interventions to have the same positive relationship between the percent changes in sex hormone concentrations and the percent change in bone mineral density of the arms.

We expected both intervention groups to see an increase in testosterone, free testosterone, and estradiol [31, 32, 52-54, 58-62, 66, 74, 75]. In weight-bearing bones (i.e. hips and legs), the additional effects of gravitational loading during the plyometric intervention will weaken the relationship between bone mineral density and the sex hormones; therefore, we expected the relationship between bone mineral density and the sex hormones to be stronger in the resistance training group. In non-weight-bearing bones (i.e. arms), we expected both intervention groups to have the same relationship between the sex hormones and bone mineral density.

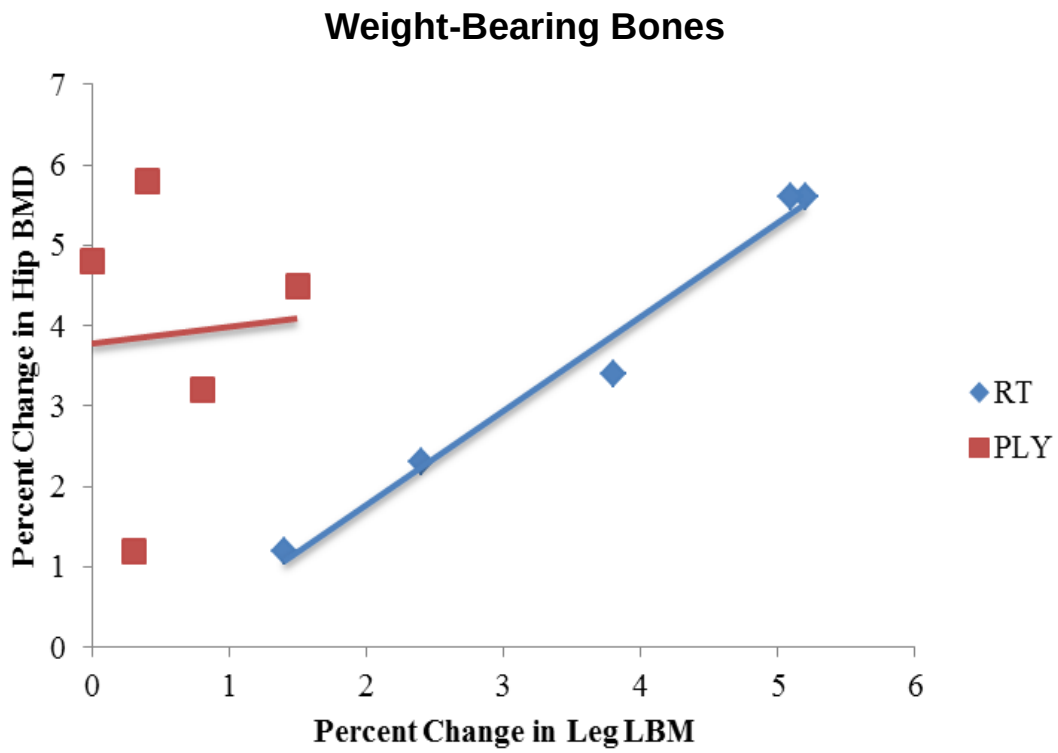


FIGURE 4. The theoretical results for hypothesis 1. We hypothesized that the RT group would increase LBM of the weight-bearing sites, and hopefully, also increase BMD at weight-bearing BMD sites. We hypothesized that the PLY group, most likely, will not significantly increase LBM of the weight-bearing sites. However, due to ground reaction forces, we expected the PLY group to increase BMD of the weight-bearing sites. Thus, we expected the relationship between the LBM of weight-bearing sites and the BMD of weight-bearing sites to be stronger in the RT group, even if the PLY group were to have greater increases in BMD. RT: resistance training; LBM: lean body mass; BMD: bone mineral density; PLY: plyometrics.

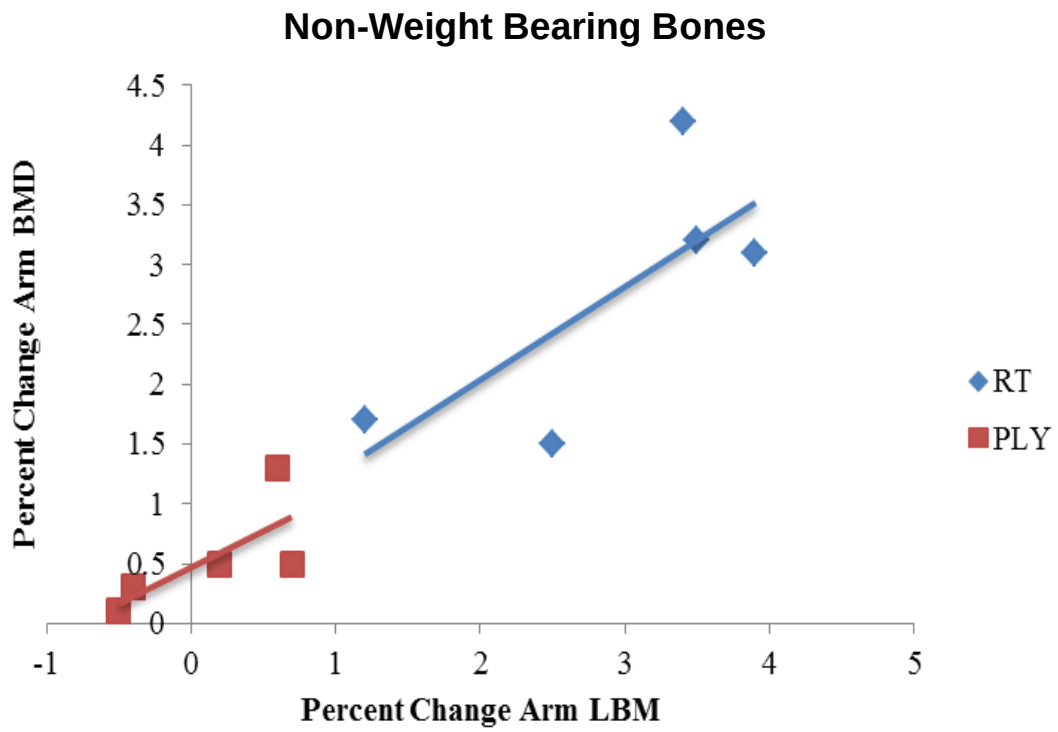


FIGURE 5. The theoretical results for hypothesis 2. Since the RT workout included the military press and the bent-over row, we expected the RT group to have a greater increase in arm LBM than the PLY group. Since ground reaction forces are not a factor for BMD of the upper body, we expected the relationship between arm LBM and arm BMD to be the same in both experimental groups. RT: resistance training; LBM: lean body mass; PLY: plyometrics; BMD: bone mineral density.

METHODS

A randomized longitudinal intervention in active, osteopenic, adult males was used to determine whether changes in lean body mass associated with a resistance training or plyometric intervention correlate with changes in bone mineral density. We also determined if resistance training or plyometric exercises are associated with a chronic increase in testosterone, free testosterone, and/or estradiol, and if the associated increase is related to an increase in bone mineral density. The study timeline is shown in **FIGURE 6.**

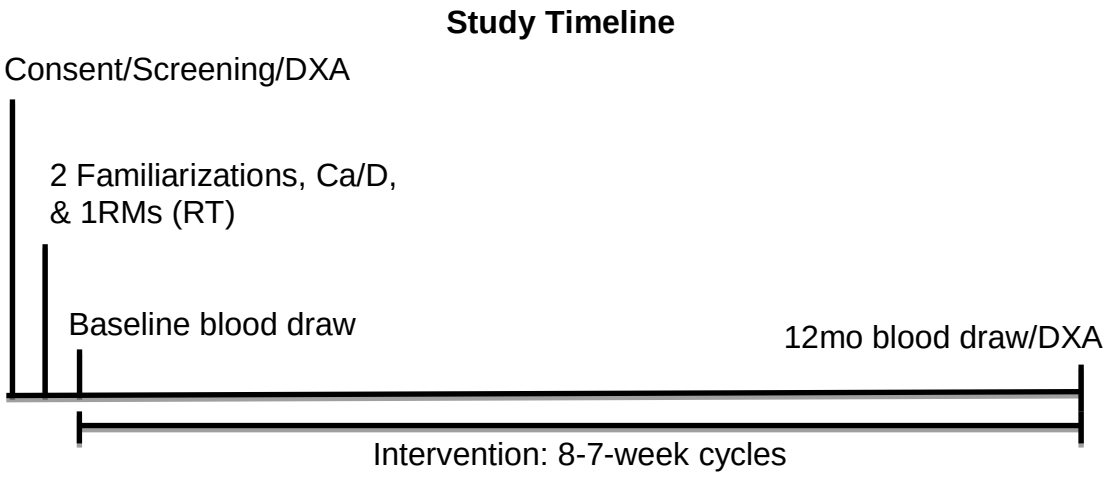


FIGURE 6. Study timeline. The protocol began with a consent and screening meeting, in which the participants were screened for eligibility, filled out questionnaires, and signed the consent form. If eligible, the participant received a DXA scan. If the participant was osteopenic (spine and/or hip BMD T-score <-1.0 and >-2.5) and chose to participate, he was randomized into either the RT or PLY group. Then, the subject had 2 familiarization sessions to get acquainted with the exercises, began taking calcium and vitamin D, and the subjects in the RT group had a 1-RM session. Once the preliminary steps were taken, the next step was to obtain a baseline blood sample, after which the intervention began. The blood draw and a DXA scan were repeated at the end of the study (12-month). DXA: dual x-ray absorptiometry scan, BMD: bone mineral density; RT: resistance training; PLY: plyometric; RM: repetition maximum.

Subjects

Twenty males between the ages of 25-60 y (mean: 42 ± 9.7y) were recruited from the University of Missouri and Columbia community through the University's Info email, local sporting goods stores, and flyers (**Appendix A**) posted on campus and on bike trails. To be eligible for this study, participants had to be between 25 and 60 years of age, participate in a minimum of 4 hours of moderate-intensity physical activity per week that does not already include either resistance training or plyometric exercises, and have osteopenia of the hip and/or spine (i.e. spine and/or hip bone mineral density t-score < -1.0 and > -2.5 [4]). The reasoning behind limiting this study to males between the ages of 25 and 60 years was to ensure that all participants are finished with the young adulthood lifecycle period at which time skeletal maturation is complete to eliminate differences between young adults and adults. The exclusion criteria included a current or previous medical condition affecting bone health, currently taking any medication that affects bone metabolism or prevents exercise, implanted metal to interfere with bone density scans, tobacco use within the last 5 years, or more than 3 alcoholic drinks per day. Current research has shown tobacco use and excessive alcohol consumption may interfere with normal bone metabolism [76]. Each subject received $1000 for completing the 12-month intervention study.

Initial screening

Interested participants reported to the Exercise Physiology Laboratory, Department of Nutritional Sciences, McKee Gymnasium for the initial screening

and to review the informed consent (**Appendix B**). Each subject was informed of the study purpose, protocol, potential risks and benefits, and reviewed the informed consent form. The subjects were informed that their participation was completely voluntary, and they were able to drop-out at any time.

Pre-testing

After the initial screening, if the subject wished to participate in the study, he signed the informed consent at the second visit. After providing written consent, each subject read and signed the HIPAA Authorization form (**Appendix C**). Then the subject filled out the medical (Physical Activity Readiness-Questionnaire), modified sports participation history (Historical Leisure Activity Questionnaire) [77], and calcium food frequency questionnaires (**Appendices D**, **E**, & **F** respectively), which were reviewed with the subject to ensure accuracy. After the questionnaires were completed and reviewed, the potential subject underwent dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500, Waltham, MA) scans of the whole body, lumbar spine, and left hip. This determined whether the potential subject has osteopenia of the spine and/or hip and met the final inclusion criterion to participate in the study. Each potential subject, whether or not they met the requirements for the study, received a copy of their baseline data. The subjects with osteopenia who are eligible for the study and consent to participate were provided with a 7-day diet record (**Appendix G**) and a 7-day physical activity log (**Appendix H**) to record their dietary intake and daily physical activity, respectively. At this point, the subject

was randomized to either the plyometric group (PLY) or the resistance training (RT) group.

Familiarization. The subject returned to the lab two more times for familiarizations, which introduced them to the 12 plyometric jumps or the 6 resistance training exercises. This was not a formal workout—just an introductory session to ensure each subject had correct form and was properly loading their bones. The plyometric jumps included the squat jump, forward hop, split squat, lateral box push-off, bounding, lateral bounding, box jump, lateral hurdle, zig-zag, single-leg lateral hurdle, jump-off the box, and depth jump. The resistance training exercises included the squat, military press, deadlift, bent-over row, lunges, and calf raises. Both the PLY and RT subjects completed two sets of 15 repetitions of abdominal exercises and low-back extensions after each workout.

Plyometric Jumps. After the familiarizations, the subject was ready to begin participation in the study. Each workout began with a 10-minute warm-up and ended with a 5-minute cool down. The PLY group came into the lab 3 times a week, each week, for the duration of the 12-month study, with a minimum of 8-hours between workouts. Each time they came in, they were supervised for safety and to ensure completion of the workout. They participated in eight 6-week cycles, with a rest week after the cycle was complete. The first two weeks of the cycle are the “light” weeks, where each participant did 10 touches of 4 different jumps, for a total of 40 touches. The third and fourth weeks of the cycle

were the “moderate” weeks, where each subject did 10 touches of 8 different jumps, for a total of 80 touches. The fifth and sixth weeks of the cycle were the “heavy” weeks, where each participant did 10 touches of 10 different jumps, for a total of 100 touches. As *jump-off the box* and the *depth jump* are the high-intensity jumps, these jumps were only done during the heavy weeks and were the last two jumps on those days (**Table 1**). In between each jump was a 10-second rest period, with the exception of the bounding-type jumps. The bounding-type jumps were performed in two sets of 5, in which the first 5 were completed consecutively, then the subject rested for 30 seconds, and then the last 5 jumps were completed. At the end of each workout, each subject completed 2 sets of 15 repetitions of both abdominal crunches and low back exercises. Prior to each periodized cycle, each subject did a vertical jump test, where the study personnel would measure and record the vertical jump height to account for changes in jumping performance.

TABLE 1. Number of touches (jumps) per week for the plyometric group.

	Light	**Moderate**	**Heavy**	**Rest**
Weeks	1-2	3-4	5-6	7
Touches	40	80	100	--

Resistance Training. The RT group came into the lab 2 times per week, each week, for the 12-month study with a minimum of 48 hours between workouts to allow for adequate muscle recovery. Similar to the PLY group, the RT group warmed-up for 10 minutes prior to the workout and cooled-down for a minimum of 5 minutes post-workout. The RT group also participated in 6-week

cycles, with a rest week after each cycle was completed. Every session was supervised to ensure safety and completion of workout. Prior to each cycle, the RT subjects completed repetition maximum (RM) testing, in which their new RM's were found and used in the following cycle. 1-RMs were determined for the squat, military press, and deadlift exercises, while 10-RMs were used for the bent-over rows, lunges, and calf raise exercises.

After completion of the RM workout, the subject was ready to begin the first, and every subsequent cycle. The first two weeks of each cycle were the "light" weeks, and each subject did a total of four sets for each exercise. The first set was 10 reps at 20% of the RM, and was the "warm-up" set. The following three sets were 10 reps at 50% of the RM. After all four sets of the squats were completed with rest between each set, the subject moved onto the military press and so forth. The third and fourth weeks were the "moderate" weeks. Once again, the subject did four sets, and the first set, or "warm-up" set, remained at 20% of the RM. The second and third sets were 10 reps at 60% of the RM, while the fourth set was 6-8 reps at 70% and 75% of the RM for the third and fourth weeks, respectively. The fifth and sixth weeks, or "heavy" weeks, followed the same workout design as the "moderate" weeks, but the last set was 3-5 reps at 80% and 90% of the RM for the fifth and sixth weeks, respectively (**Table 2**). After both the PLY and RT groups finished their first cycle, they took a one-week break and reported back to the lab the following week to start the next 6-week cycle.

Anthropometric Data

Body weight (to the nearest 0.5 kg), height (to the nearest 0.5 cm), body mass index, and percent body fat were measured at baseline and 12 months. The height and weight measurements were used to calculate the subjects' body mass index (kg/m^2). Percent body fat was determined using the whole body DXA scan.

TABLE 2. Percentage of RM's for resistance training group.

	Weeks 1-2 Light	**Weeks 3-4 Moderate**	**Weeks 5-6 Heavy**	**Week 7 Rest**
Set 1	20% RM	20% RM	20% RM	--
Set 2	50% RM	60% RM	60% RM	--
Set 3	50% RM	60% RM	60% RM	--
Set 4	50% RM	70/75% RM*	80/90% RM*	--

*The relative weights for the first and second weeks of both moderate and heavy.

Questionnaires

Each participant completed a medical history questionnaire, a modified Historical Leisure Activity Questionnaire [77], and a Calcium Food Frequency Questionnaire. The responses on theses questionnaires were reviewed with study personnel to ensure accuracy of information. At baseline (0 month), 3, 6, 9, and 12 month intervals, each subject completed a 7-day food diary and 7-day physical activity log to monitor changes in nutrient intake or physical activity. The foods from the 7-day food diary were entered into Food Processor 8.0 (Food Processor 8.0, Salem, OR) to assess the daily nutrient intake for each participant. The physical activity log required the participant to record the activity

type, intensity, duration, and frequency. The Compendium of Physical Activities - was used to estimate the daily energy expenditure during exercise.

Calcium and vitamin D supplementation

Calcium and vitamin D are essential for normal bone health [78], and the dietary intake of these nutrients in the general population and the athletic population is sub-optimal [79]. It is recommended that men with or at risk for osteoporosis consume 1000-1200 mg calcium daily [22]. To ensure the subjects in this study were getting an adequate intake of these nutrients, they were supplied with calcium and vitamin D supplements containing 600 mg calcium and 200 I.U. of vitamin D per tablet (*Nature Made,* Mission Hills, CA). Each subject took two tablets daily, preferably one in the morning and one in the evening. To ensure the vitamins were taken daily, the tablets were dispensed in bottles of 120, which lasted 60 days, and recorded in each subjects' log book.

Bone mineral content and density scans

DXA (Hologic QDR 4500, Waltham, MA) was used to measure bone area, bone mineral content, and bone mineral density of the whole body, total left hip, arms and legs at baseline (0 month), 6 months, and 12 months. Scans of the whole body and left hip were used for analysis. Left leg and left arm bone area, bone mineral content, and bone mineral density were taken from the whole body scan. Areal bone mineral density (g/cm^2) was calculated from bone area (cm^2) and bone mineral content (g) by the software supplied with DXA scanner. Normal bone mineral density is defined as a t-score of >-1.0 standard deviations,

osteopenia is defined as having a t-score < 1.0 standard deviations and >-2.5 standard deviations, while osteoporosis is categorized as having bone mineral density ≤ -2.5 standard deviations of either the spine or hip [4]. Body composition was measured using the whole body scan DXA scan.

Serum measures of sex-hormones

Blood samples were collected at baseline (0 months) and 12 months from an antecubital vain by a trained phlebotomist. At each time point, the blood sample was taken after an overnight fast (at least 10 hours) and drawn between the hours of 6:00 and 8:00am. At each time point, the blood samples were taken at approximately the same time to account for any diurnal variations. Blood was dispensed into plasma separator tubes containing EDTA, where it sat for 20 minutes. The blood was then centrifuged at 2000 rpm for 15 minutes at 0 ºC. The plasma was removed from the EDTA tube and immediately frozen at -80 ºC. The concentrations of testosterone, estradiol, and SHBG were determined using commercially available ELISA kits (Testosterone: Testosterone Parameter Assay Kit; R&D Systems, Minneapolis, MN; Estradiol: Estradiol ELISA; BQ Kits, San Diego, CA; SHBG: Sex Hormone Binding Globulin (SHBG) ELISA; ALPCO Diagnostics, Salem, NH). We used two different equations to determine the proportions of free testosterone. Equation 1 was used for those with low total testosterone levels (<5 nmol/L), and equation 2 was used for those with high total testosterone levels (>5 nmol/L) [80].

Equation 1 (total testosterone < 5 nmol/L):

FT (pmol/L) = -6.593 + 19.304*TT + 0.056*SHBG - 0.0959*TT*SHBG

Equation 2 (total testosterone > 5 nmol/L):

FT (pmol/L) = -52.65 + 24.4*TT – 0.704*SHBG – 0.0782*TT*SHBG – $0.0584*TT^2$

where TT is the concentration of total testosterone (nmol/L) and SHBG is the concentration of SHBG (nmol/L).

Statistical analysis

Normality was checked for each variable using the Kolmogorov-Smirnov test, and a visual check of histograms and Q-Q plots to ensure a bell-shaped curve and a linear relationship, respectively. Descriptive statistics were performed on demographic and anthropometric data. Due to an invalid baseline scan in one RT subject, only 9 hip bone mineral density values were used for analysis. Also, due to large variations in hormone concentrations among the duplicates, 3 RT subjects and 1PLY subject was left out of the SHBG analyses, 2 PLY subjects were left out of the total testosterone analyses, 3 RT subjects and 4 PLY subjects were left out of the free testosterone analyses, and 2 RT subjects and 1 PLY subject was left out of the estradiol analyses.

A two-way (group and time) repeated-measures ANOVA was performed to compare the effects of plyometric versus resistance training exercise on whole body and regional (upper/lower body) lean body mass and bone mineral density. Main effects were considered statistically significant at a p-value < 0.05, and interaction effects were considered statistically significant at a p-value < 0.1. As

appropriate, age, height, weight, and body mass index were added as covariates. Post hoc paired samples T-tests were performed for significant main interaction effects (group x time). The relationship between percent changes in lean body mass and bone mineral density and sex hormones and bone mineral density were assessed independently in resistance training and plyometric interventions using one-tailed Pearson's correlations. Correlations were considered statistically significant at a p-value < 0.05. One-tailed correlations were used because we expected one-directional changes; i.e. $r>0$.

RESULTS

A total of 20 subjects completed the 12-month intervention (PLY: n=10; RT: n=10). With the exception of weight and body mass index, there were no significant differences in baseline characteristics between groups (**Table 3**). On average, the RT group weighed 16.1 kg more than the PLY group and had a body mass index that was 5 kg/m^2 greater than the PLY group. The RT group increased strength as measured by the 1 RMs and 10 RMs, while the PLY group increased their vertical jump performance over the one-year intervention (**Table 4**). There were no changes in diet or physical activity energy expenditure outside of the exercise intervention in either group (**Appendix I**).

Changes in Bone Mineral Density and Lean Body Mass

The percent changes from baseline to post-intervention in bone area were less than 2% for each subject's whole body and hip scans (data not shown). Group means and standard errors for whole body, left leg, left arm and hip bone mineral densities are shown in **Table 5.** After the 12-month RT or PLY intervention, both intervention groups had a significant increase in whole body bone mineral density with a main effect for time (p= 0.025) (**Figure 7**). Leg bone mineral density increased in both groups, controlling for BMI (main effect for time, p= 0.024) (**Figure 8**). In regards to hip bone mineral density, there was a significant interaction effect (group x time, p= 0.068), such that the RT group increased and the PLY group remained the same (**Figure 9**). There were no statistically significant changes in left arm bone mineral density.

Group means and standard deviations for whole body, left leg, and left arm lean body masses are shown in **Table 5.** There was a significant interaction effect (group x time, p= 0.080) for left arm lean body mass, such that the PLY group decreased left arm lean body mass and the RT group stayed the same (**Figure 10**). There were no statistically significant changes in whole body or left leg lean body mass in either intervention group.

Relationship between Percent Changes in Bone Mineral Density of Weight-Bearing Sites and Percent Changes in Lean Body Mass

The correlation coefficients and significance values for the correlations between weight-bearing lean body mass sites (whole body and left leg) and weight-bearing bone mineral density sites (whole body, left leg, and hip) can be seen in **Table 6**. There was a significant negative relationship between the percent change in whole body lean body mass and the percent change in left leg bone mineral density in RT group, but a significant positive relationship between the percent change in whole body lean body mass and the percent change in left leg bone mineral density in the PLY group (RT: r= -0.577, p= 0.040; PLY: r= 0.581, p= 0.039) (**Figure 11**). There was a significant positive relationship between the percent change in whole body lean body mass and the percent change in hip bone mineral density from baseline to 12 months in the PLY group (r= 0.565, p= 0.044) (**Figure 12**). There were no other significant relationships between the percent change in the bone mineral density of weight-bearing sites and the percent change in lean body mass of the lower body.

TABLE 3. Anthropometrics at 0 and 12 months.

	Resistance Training (n=10)		**Plyometric** (n=10)	
	0 month	**12 month**	**0 month**	**12 month**
Age (y)	42.1 ± 3.15	--	41.9 ± 3.16	--
Height (m)	1.78 ± 0.02	--	1.77 ± 0.01	--
Weight (kg)	88.9 ± 5.08	86.4 ± 12.6	72.8 ± 3.32*	86.8 ± 5.4
Body Mass Index (kg/m^2)	28.1 ± 1.45	28.4 ± 4.2	23.1 ± 1.05*	26.4 ± 1.5
Body Fat (%)	20.6 ± 1.53	19.5 ±1.5	20.6 ± 1.71	20.3 ±1.6
Lean Mass (kg)	64.8 ± 3.2	65.7 ± 3.4	58.2 ± 1.8	58.3 ± 19.4
Fat Mass (kg)	19.2 ± 2.1	25.0 ± 6.5	16.3 ± 1.8	14.6 ± 1.7

*Significantly different from the RT group at 0 months.
Data are means ± S.E.

TABLE 4. Repetition maximum and vertical jump values at 0 and 12 months.

		0 Month	**12 Month**	**Percent Change**
RT	**RMs** (lbs)			
	Squat (1 RM)	194 ± 12	353 ± 16*	86 ± 11
	Military Press (1 RM)	96 ± 7	136 ± 8*	45 ± 9
	Dead Lift (1 RM)	199 ± 21	327 ± 20*	74 ± 13
	Bent-Over Row (10 RM)	110 ± 6	157 ± 7*	45 ± 8
	Forward Lunges (10 RM)	84 ± 9	170 ± 9*	118 ± 25
	Calf Raises (10 RM)	181 ± 14	313 ± 18*	77 ± 8
PLY	**Vertical Jump** (in)	18.2 ± 1.4	19.4 ± 1.3	7.8 ± 5.6

*Significantly different from 0 month values.
Data are means ± S.E.

Relationship between the Percent Change in Bone Mineral Density of Non-Weight-Bearing Sites and the Percent Changes in Lean Body Mass of the Upper Body

The correlation coefficients and significance values for the correlations between the percent change in bone mineral density of non-weight-bearing sites (left arm) and the percent change in lean body mass of the left arm can be seen in **Table 7.** There was a significant positive relationship between the percent change in left arm lean body mass and left arm bone mineral density in the RT group, but not in the PLY group (RT: r= 0.577, p= 0.047; PLY: r= 0.189, p= 0.300) (**Figure 13**).

Repeated measures ANOCOVAs were ran for the whole body, left leg, left arm, and hip bone mineral density sites using the following covariates: 1) energy expenditure/body weight (kg) at 0 months, 2) energy expenditure/body weight (kg) at 12 months, and 3) the percent change in energy expenditure/body weight (kg) (see **Appendix J**). In the RT group, the percent change in squat 1-RM was used as a covariate for the whole body, left leg, and hip bone mineral density sites, and the percent change in military press 1-RM was used as a covariate for the left arm bone mineral density site. In the PLY group, the percent change in vertical jump was used as a covariate for the whole body, left leg, left arm, and hip bone mineral density sites (see **Appendix K**).

Correlations were ran between the percent change in energy expenditure/body weight and the percent change in whole body, left leg, left arm, and hip bone mineral density (data not shown). There was a positive relationship between the percent change in energy expenditure/body weight and the percent

change in hip bone mineral density in the PLY group (PLY: r= 0.633, p= 0.034; RT: r= -0.714, p= 0.088). In the RT group, correlations were ran between the percent change in squat 1-RM and the percent change in whole body, left leg, and hip bone mineral density (data not shown). There was a positive relationship between the percent change in the squat 1-RM and left leg bone mineral density (r= 0.605, p= 0.042). Also in the RT group, a correlation was ran between the percent change in military press 1-RM and the percent change in left arm bone mineral density (data not shown). It was not significant. In the PLY group, correlations were ran between the percent change in vertical jump height with the percent change in whole body, left leg, left arm, and hip bone mineral density (data not shown). There was a positive relationship between the percent change in vertical jump height and the percent change in hip bone mineral density (r= 0.756, p= 0.006).

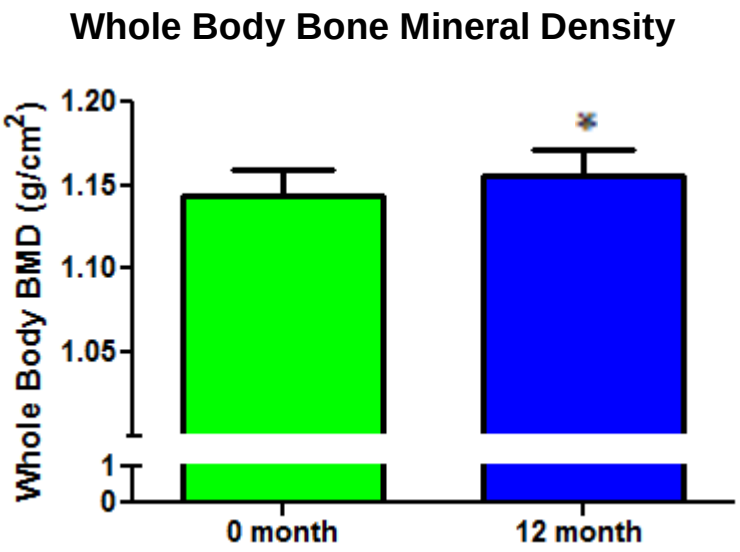


FIGURE 7. In both intervention groups, there was a significant increase from baseline to post-intervention in whole body bone mineral density (main effect for time, p= 0.025).
*Significantly different from 0 month.

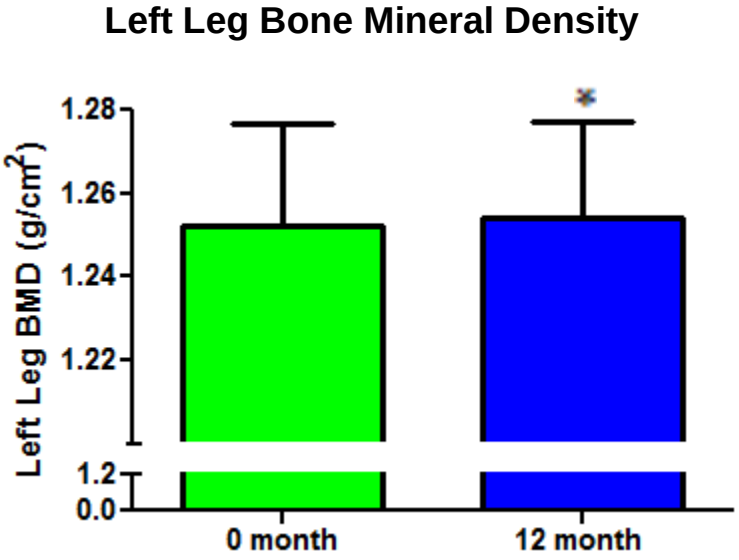


FIGURE 8. In both interventions, there was a significant increase from baseline to post-inervention in left leg bone mineral density (main effect for time, p= 0.024).
*Significantly different from 0 month.

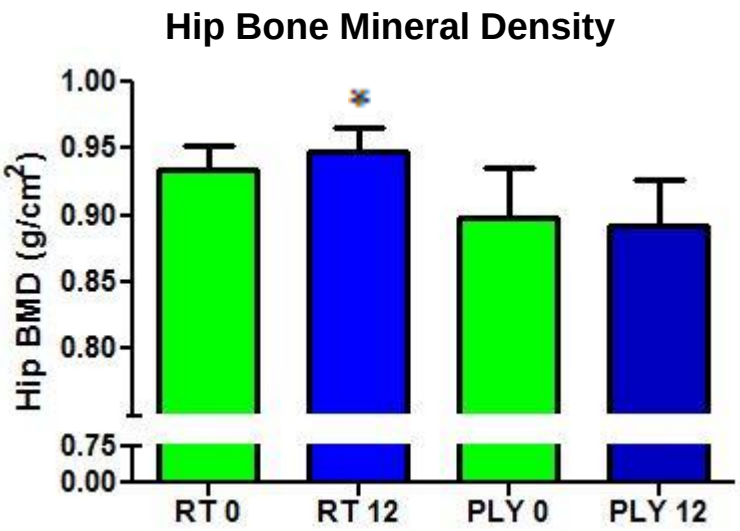


FIGURE 9. The RT group significantly increased hip bone mineral density from baseline to post-intervention, while the PLY group did not change (interaction effect (group x time), p= 0.068).
*Significantly different from 0 month within group.

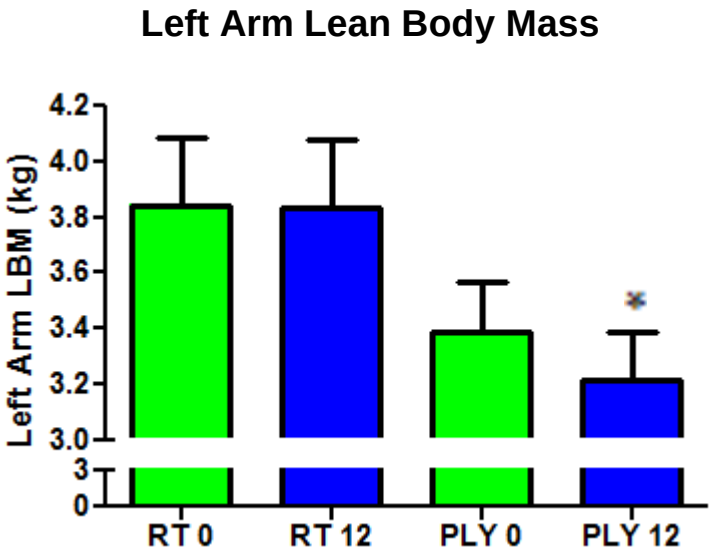


FIGURE 10. The PLY group significantly decreased left arm lean body mass from baseline to post-intervention, while the RT group did not change (interaction effect (group x time), p= 0.080).
*Significantly different from 0 month within group.

Relationship between Whole Body Lean Body Mass and Left Leg Bone Mineral Density

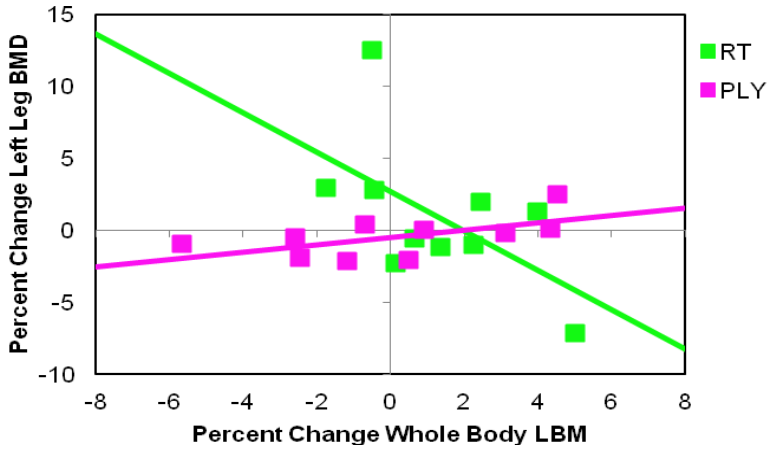


FIGURE 11. There was a significant positive relationship between the percent change in whole body lean body mass and the percent change in left leg bone mineral density in the PLY group, and a significant negative relationship between the percent change in whole body lean body mass and the percent change in left leg bone mineral density in the RT group (PLY: r= 0.581, p= 0.039; RT: r= -0.577, p= 0.040).

Relationship between Whole Body Lean Body Mass and Hip Bone Mineral Density

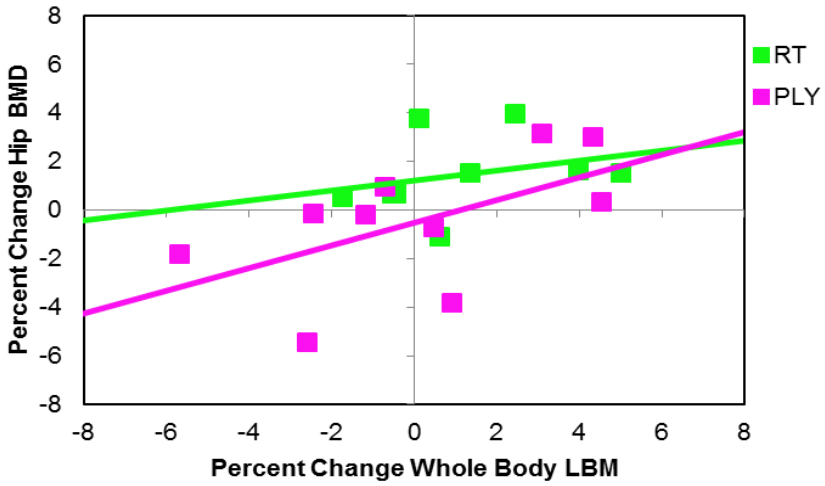


FIGURE 12. There was a significant positive relationship between the percent change in whole body lean body mass and the percent change in hip bone mineral density in the PLY group (PLY: r= 0.565, p= 0.044; RT: r= 0.285, p= 0.229).

Relationship between Left Arm Lean Body Mass and Left Arm Bone Mineral Density

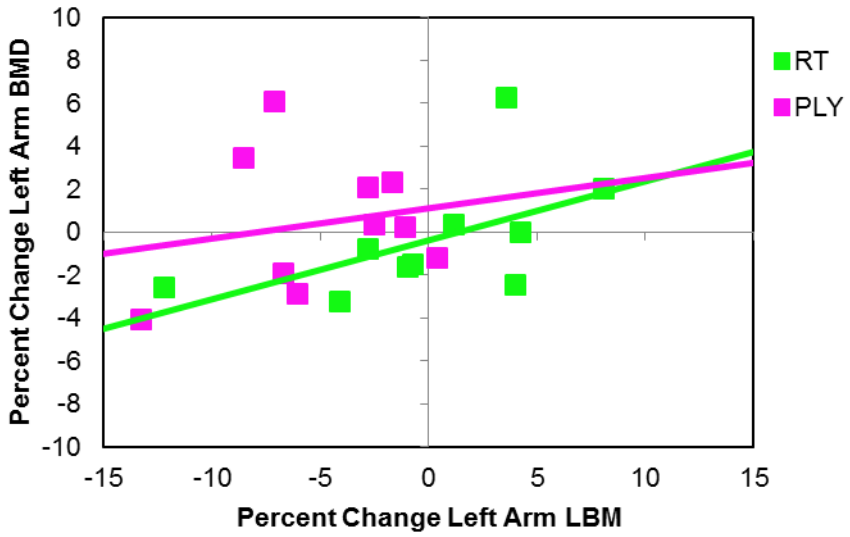


FIGURE 13. There was a significant positive relationship between the percent change in left arm lean body mass and the percent change in left arm bone mineral density in the RT group (RT: r= 0.577, p= 0.047; PLY: r= 0.189, p= 0.300).

TABLE 5. Site-specific bone mineral density and lean body mass at 0 and 12 months.

		0 Month	12 Month	Percent Change
Whole Body				
BMD (g/cm^2)†	RT	1.174 ± 0.019	1.191 ± 0.020	1.47 ± 0.70
	PLY	1.115 ± 0.019	1.121 ± 0.020	0.50 ± 0.44
LBM (kg)†	RT	64.8 ± 3.2	65.7 ± 3.4	1.31 ± 0.7
	PLY	58.2 ± 1.8	58.3 ± 1.9	0.08 ± 1.0
Left Leg				
BMD (g/cm^2)†	RT	1.269 ± 0.028	1.281 ± 0.030	0.93 ± 1.59
	PLY	1.235 ± 0.028	1.227 ± 0.030	-0.47 ± 0.45
LBM (kg)	RT	10.6 ± 0.6	10.5 ± 0.7	-1.3 ± 1.6
	PLY	9.6 ± 0.4	9.7 ± 0.4	1.0 ± 1.0
Left Arm				
BMD (g/cm^2)	RT	0.853 ± 0.017	0.849 ± 0.017	-0.36 ± 0.89
	PLY	0.789 ± 0.017	0.793 ± 0.017	0.45 ± 0.98
LBM (kg)**	RT	3.8 ± 0.2	3.8 ± 0.2	0.0 ± 1.8
	PLY	3.4 ± 0.2	3.2 ± 0.2*	-4.9 ± 1.3
Hip				
BMD (g/cm^2)**	RT	0.934 ± 0.031	0.947 ± 0.029*	1.46 ± 0.53
	PLY	0.898 ± 0.030	0.892 ± 0.027	-0.47 ± 0.86

Data are means ± S.E.

*Significantly different from baseline values at the $p<0.05$ significance level. †Significant main effect for time (see Figures 7, 8, and 10). **Significant group by time interaction (see Figure 9 and 11).

BMD: bone mineral density, LBM: lean body mass.

TABLE 6. The relationship between weight-bearing lean body mass sites (whole body and left leg) and weight-bearing bone mineral density sites (whole body, left leg, and hip).

		%Δ WB BMD	%Δ LL BMD	%Δ Hip BMD
%ΔWB LBM	RT (n=10)	r= -0.293 p= 0.206	r= -0.577* p= 0.040	r= 0.285 p= 0.229
	PLY (n=10)	r= 0.119 p= 0.372	r= 0.581* p= 0.039	r= 0.565* p= 0.044
%Δ LL LBM	RT (n=10)	--	r= -0.022 p= 0.476	r= -0.124 p= 0.376
	PLY (n=10)	--	r= -0.064 p= 0.431	r= 0.516 p= 0.063

*Significant at the p ≤ 0.050 significance level (one-tailed).
%Δ: percent change, WB: whole body; LL: left leg; BMD: bone mineral density, LBM: lean body mass.

TABLE 7. The relationship between the non-weight-bearing lean body mass site (left arm) and the non-weight-bearing bone mineral density site (left arm).

		%Δ Left Arm LBM
%Δ Left Arm BMD	RT (n=10)	r= 0.557* p= 0.047
	PLY (n=10)	r= 0.189 p= 0.300

*Significant at the p ≤ 0.050 significance level (one-tailed).
%Δ: percent change, BMD: bone mineral density, LBM: lean body mass.

Hormonal Changes

All measured hormone concentrations were within the recommended reference ranges. After the 12-month RT or PLY intervention, there were no significant changes in SHBG, testosterone, free testosterone, or estradiol concentrations in either group (**Table 8**). Free testosterone was calculated from total testosterone and SHBG concentrations, and there were no significant changes. Even after adjusting for covariates, i.e. age, weight, height, and body mass index, there were no significant changes in any of the hormones (data not shown).

Relationship between the Percent Changes in Hormone Concentrations and the Percent Changes in Bone Mineral Density

The correlation coefficients and significance values for the relationship between the percent changes in the hormone concentrations (SHBG, testosterone, free testosterone, and estradiol) and the percent changes in bone mineral density at the whole body, left leg, left arm, and hip for RT and PLY are in **Table 9**. In both the RT and PLY groups, there was a significant negative relationship between the percent change in total testosterone and the percent change in hip bone mineral density (RT: r= -0.614, p= 0.039; PLY: r= -0.643, p= 0.043) (**Figure 14**). There was also a significant negative relationship between the percent change in estradiol and the percent change in hip bone mineral density in the RT group (RT: r= -0.735, p= 0.030) (**Figure 15**). There were no

other significant relationships between the measured sex hormones and the bone mineral density sites.

The correlation coefficients and significance values for the relationships between the percent change in the hormone concentrations (SHBG, testosterone, free testosterone, and estradiol) and the percent changes in lean body mass at the whole body, left leg, and left arm for RT and PLY can be found in **Appendix L.**

TABLE 8. Hormone concentrations at 0 and 12 months and reference ranges for adult men*.

Hormones	Group	0 Month	12 Month	Percent Change	Reference Ranges
SHBG	RT (n=7)	88.3 ± 24.2	52.3 ± 11.7	-16.0 ± 18.7	7-70
(nmol/L)	PLY (n=9)	69.1 ± 18.4	58.6 ± 15.0	-2.6 ± 19.7	
Total Testosterone	RT (n=10)	21.7 ± 2.7	19.0 ± 2.7	-0.3 ± 15.4	12.72-38.16
(nmol/L)	PLY (n=8)	20.6 ± 4.3	18.5 ± 1.7	3.9 ± 12.2	
Free Testosterone	RT (n=7)	265.4 ± 78.2	201.0 ± 32.6	-67.5 ± 99.0	115.83-386.09
(pmol/L)	PLY (n=6)	303.7 ± 99.8	284.4 ± 50.4	-104.88 ± 77.0	
Total Estradiol	RT (n=8)	11.1 ± 3.4	11.2 ± 3.7	17.6 ± 51.6	10-50
(pg/ml)	PLY (n=9)	21.8 ± 8.7	14.0 ± 5.8	-38.5 ± 31.9	

Data are means ± S.E.

There are no significant changes in either group.

*Reference ranges for SHBG, total testosterone, and total estradiol were taken from the ELISA kits; reference range for free testosterone taken from the Mayo Clinic [81].

TABLE 9. The relationship between the percent change in sex hormone concentrations (sex hormone binding globulin, testosterone, free testosterone, and estradiol) and the percent change in bone mineral density at the whole body, left leg, left arm, and hip.

		%Δ Whole Body BMD	%Δ Left Leg BMD	%Δ Left Arm BMD	%Δ Hip BMD
%Δ SHBG	RT (n=7)	r= 0.525 p= 0.113	r= 0.374 p= 0.204	r= -0.511 p= 0.121	r= 0.542 p= 0.104
	PLY (n=9)	r= -0.195 p= 0.308	r= 0.336 p= 0.188	r= -0.379 p= 0.157	r= 0.153 p= 0.348
%Δ Testosterone	RT (n=10)	r= -0.125 p= 0.366	r= -0.115 p= 0.376	r= 0.115 p= 0.376	r= -0.614* p= 0.039
	PLY (n=8)	r= 0.406 p= 0.159	r= -0.280 p= 0.251	r= 0.065 p= 0.439	r= -0.643* p= 0.043
%Δ Free Testosterone	RT (n=7)	r= 0.173 p= 0.355	r= 0.480 p= 0.138	r= -0.378 p= 0.202	r= -0.354 p= 0.218
	PLY (n=6)	r= 0.112 p= 0.416	r= 0.094 p= 0.430	r= 0.559 p= 0.124	r= -0.290 p= 0.288
%Δ Estradiol	RT (n=8)	r= -0.280 p= 0.251	r= 0.150 p= 0.362	r= -0.380 p= 0.177	r= -0.735* p= 0.030
	PLY (n=9)	r= -0.167 p= 0.334	r= 0.226 p= 0.279	r= 0.024 p= 0.476	r= -0.440 p= 0.118

*Significant at the p≤0.050 significance level (one-tailed).
BMD=bone mineral density; LBM=lean body mass; %Δ= percent change.

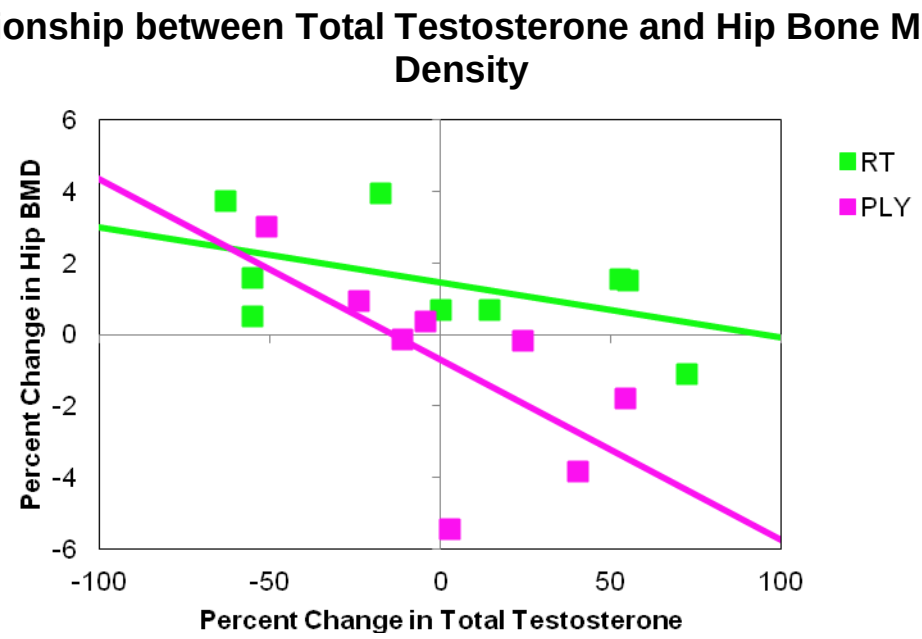


FIGURE 14. There was a significant negative relationship between the percent change in total testosterone and the percent change in hip bone mineral density in both the RT and PLY groups (RT: r= -0.614, p= 0.039; PLY: r= -0.643, p= 0.043).

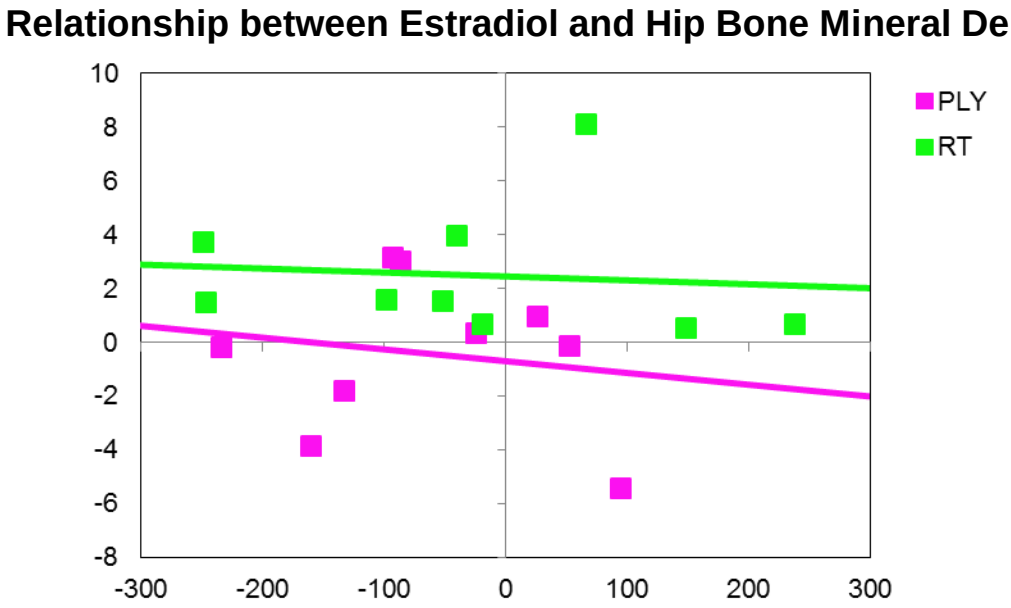


FIGURE 15. There was a significant negative relationship between the percent change in total estradiol and the percent change in hip bone mineral density in the RT group (RT: r= -0.735, p= 0.030; PLY: r= -0.440, p= 0.118).

DISCUSSION

Our objectives were to determine if 12 months of plyometric or resistance training exercise increases or maintains bone mineral density in active osteopenic men, and whether the maintenance or increase in bone mineral density is associated with changes in lean body mass, testosterone or estradiol concentrations. This study is the first to compare the effects of resistance training and plyometric exercises on men with osteopenia. There is limited literature on exercise studies and prescriptions for men with low bone mineral density, and there is a lack of research that examines the osteogenic response of plyometric jump training on osteopenic males. To our knowledge, only a few studies have examined plyometric activity as an exercise prescription for men [11, 34]. Guadalupe-Grau et al. [11] had a sample of both men and women, who participated in both the RT and PLY exercises, 3 times per week, for 9 weeks. They found an increase in lumbar spine and whole body bone mineral content in both men and women; however, there was a trend for a greater bone mineral density increases in men [11]. Rantalainen et al. [34] did not see a change in bone resorption or formation markers in elderly men (average age of 72 years) following 12 weeks of bilateral hopping exercise (5-7 sets at 75-90% intensity, 3 times/week); however, they did see acute biochemical changes in younger men.

The present study focused on an exercise-based treatment program with supplemental calcium and vitamin D as a means for maintaining or increasing bone mineral density in osteopenic men. The 1.5% increase in hip bone mineral

density in the RT group, the 1% increase in whole body bone mineral density in both groups, and the 4.5% increase in left leg bone mineral density in both groups are all clinically significant findings. The current literature suggests that any increase in bone mineral density is significant and will decrease fracture risk, and the incidence of fracture is not independent of the magnitude of bone mineral density increase [3]. Thus, as long as bone mass is not decreasing, the risk of fracture is lowered.

However, anti-resorptive drug therapy is another treatment option. The anti-resorptive drug therapies have been found to reduce fracture risk in post-menopausal women [89] and increase bone mineral density of the hip by 2.5% [90]. Reid et al. [91] reported a 3.1–3.5% increase in femoral neck bone mineral density after 1-year administration of zoledronic acid, the strongest bisphosphonate on the market [91]. Other literature has reported bone mineral density increases at the femoral neck, trochanter, and whole body by 5.9%, 7.8%, and 2.5%, respectively [92]. These anti-resorptive drugs prevent the loss of bone mass by promoting apoptosis of osteoclasts; thus, bone breakdown is slowed, while bone formation continues [93]. As with any drug use, there are always side-effects, and these particular drugs include side effects such as unusual fractures in the shaft of the thigh bone [94]. The atypical fractures associated with bisphosphonate administration may be due to delayed bone turnover [94]. When osteoclasts are not absorbing the weak parts of the bone, small micro-cracks are unable to heal, and they eventually cause fractures [94].

Thus, exercise as a treatment option is a cost-effective and safe means for promotion of bone mass.

The majority of studies that aim to influence bone metabolism via exercise have focused on women [2, 3, 12, 82-86] or experimental animals [33, 38-40]. A few studies have looked at both men and women [9-11], but only a handful have focused on men [24, 87, 88]. Due to differences in body weight, body composition, and hormones in men and women, the present study helps to establish an exercise prescription for men with low bone mineral density.

Bone mineral density at weight-bearing sites

Weight-bearing bones (i.e. legs and hips) transmit the body weight to the ground and in turn experience ground reaction forces, as opposed to non-weight-bearing bones (i.e. arms) that do not experience ground reaction forces. The literature points out that athletes who participate in activities that stress bone in a variety of directions, such as volleyball and gymnastics, have higher bone mineral densities than athletes in sports that are associated with only one direction of movement, like running [2]. The current study purposefully included 12 different plyometric jumps that were intended to stress bone in a variety of directions. Since the PLY group had the influence of lean body mass and ground reaction forces imposing demands on the bones, it was expected that the PLY group would see greater bone mineral density changes than the RT group.

The results of the present study suggest that dynamic weight-bearing exercise as a whole may aid in maintaining or increasing bone mineral density.

With a main effect for time, both intervention groups experienced a 1% increase in whole body bone mineral density and a 4.5% increase in left leg bone mineral density ($p= 0.025$ and $p= 0.024$, respectively). Hip bone mineral density significantly increased 1.5% in the RT group, while the PLY group remained the same (group x time, $p= 0.068$). Of the sites measured in the present study, the bone mineral densities did not decrease, as occurs with normal aging in men [95]; thus, these interventions may help increase and/or maintain bone mineral density in men. Prior to the present study, our lab group performed a 3-year follow-up study to determine the normal bone mineral density changes in active, osteopenic, adult males [96]. Over the 3-year period, bone mineral density of the arm, left hip, femoral neck, and Ward's triangle decreased by 3.3%, 2.7%, 3.5%, and 8.1%, respectively [96]. Thus, in this population, even the maintenance of bone mineral density may be a significant finding.

It is important to note that the hip is one of the two common clinically significant sites measured to diagnose osteoporosis and osteopenia. Thus, the 1.5% increase in hip bone mineral density in the RT group has clinical significance, may decrease fracture risk, and may suggest bone-loading exercise as a viable treatment for osteopenia in men.

Bone mineral density at non-weight-bearing sites

The present study paid special attention to the non-weight-bearing bones (i.e. arms). The literature has shown that both plyometric exercise [97] and resistance training exercise [84, 98] induce changes in bone morphology in a

site-specific manner. With that being said, the RT group did military press and bent-over rows; thus, we expected their upper body lean body mass and bone mineral density to increase in this group. On the contrary, the PLY group only did lower body jumping exercises, and therefore, their upper body lean body mass and bone mineral density, in theory, should not have changed. It was important to analyze the upper body lean body mass and bone mineral density, because a lot of research on osteogenic exercise uses resistance training as the intervention method. We did not see a statistically significant change in left arm lean body mass or bone mineral density in the RT group, but we did see a significant positive correlation between left arm bone mineral density and left arm lean body mass. This suggests that resistance training may be an important part of bone health in osteopenic men. Upper body plyometric exercises are not feasible for the average population; thus, the positive correlation between left arm bone mineral density and left arm lean body mass suggests that the upper body resistance exercise, with the associated increases in strength and muscular contractions, may be osteogenic.

The current research has found that over the course of a lifetime, both genders will lose between 20-30% of cancellous bone and 20-30% of cortical bone mass (**Figure 16**) [99]. Specific to the hip, men and women, on average, lose about 0.5% of hip bone mass a year [100]. Thus, the maintenance of bone mass may be a significant finding. Thus, the changes in bone mineral density in

the present study are a promising finding, and show that weight-bearing exercise has a beneficial impact on bone mass.

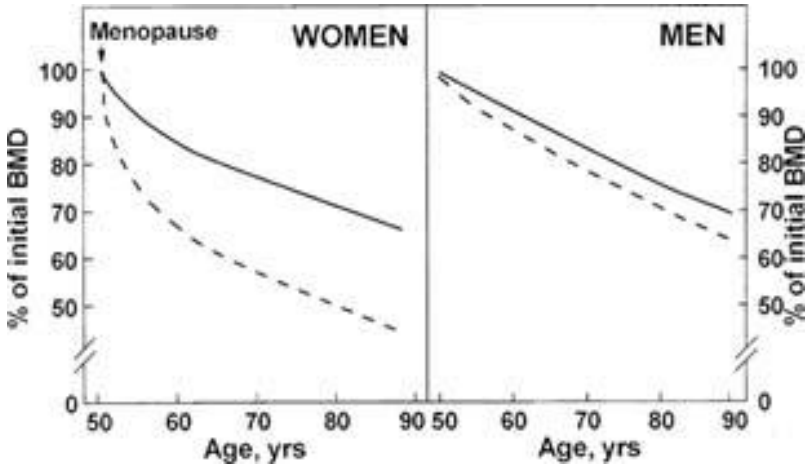


FIGURE 16. Rate of bone loss over life in cancellous (broken line) and cortical (solid line) in women (left) and men (right) from age 50 onward. Adapted from Riggs et al. [99].

Whole body and regional lean body mass

We hypothesized that the RT group would see greater increases in lean body mass than the PLY group. While we did not see any significant increases in lean body mass in the RT group, or even the PLY group, there was a significant interaction effect (group x time, p= 0.080) for left arm lean body mass, such that the RT group remained the same while the PLY group experienced a decrease in left arm lean body mass. There was no other significant group or time effects for the lean body mass sites measured.

It is well-known that RT increases lean body mass; thus, the lack of significant changes in lean body mass for the RT group was surprising. One repetition maximums (1 RMs) were performed at the start of each cycle, to

determine both the load for each workout in that cycle and to measure strength increases. With that being said, the 1 RM's increased nearly every cycle; therefore, strength increased over the duration of the study (**Table 4**). Thus, even though lean body mass did not significantly increase in the RT group, there were increases in strength. The lack of an increase in lean body mass may be due to the type of exercise these men participate in, in addition to the exercise intervention. Many of the men in this study were either runners or cyclists. Thus, staying lean may be advantageous for aerobic activities. Previous studies have found that the combination of strength and endurance training may suppress some of the strength training adaptations [101, 102]. Bouchla et al. [102] reported similar results to the present study, in that the addition of resistance training to aerobic training induced muscle strength gains, but not muscle mass gains.

Relationship between bone mineral density sites and lean body mass

It is well-known that muscle disuse causes muscle wasting and bone loss, and conversely, physical activity increases muscle strength and, potentially, bone mass [103]. While muscle strength is not independent of body weight or physical activity, the muscular contractions exert forces on bone that are greater than the gravitational forces associated with body weight [6, 103]. Thus, osteogenic exercise, such as resistance training and plyometric activity, cause strong muscular contractions that apply force to the bone and induce changes in bone mineral density [14]. Since the primary forces applied to bones are the result of

muscular contractions [103], it is expected that strength and lean body mass are positively associated with bone mass. We did find a positive significant relationship between the percent change in squat 1-RM and leg bone mineral density in the RT group ($r= 0.605$, $p= 0.042$). Thus, this finding supports increases in strength as a potential factor for positive changes in bone strength.

As far as plyometric jumps are concerned, three factors potentially contribute to an increase in bone mineral density: an increase in muscle contractile forces, an increase in body weight, and ground reaction forces. Plyometric exercises induce forces that are 3-4x the body weight of an individual [2], and research has shown that the force produced by jumping is sufficient enough to induce gains in bone mineral density [2]. Resistance training does not induce high ground reaction forces, but it can potentially increase body weight through the increase in lean body mass, which in turn generates stronger muscle contractions onto the bone. With that being said, while we did expect to see greater lower body bone mineral density increases in the PLY group, we had expected to see a stronger relationship between lower body lean body mass and lower body bone mineral density in the RT group.

As stated, we expected to see a stronger relationship between lower body lean body mass and lower body bone mineral density in the RT group. This was not necessarily the case. In the RT group, there was a negative relationship between the percent change in whole body lean body mass and the percent change in left leg bone mineral density ($r= -0.577$, $p= 0.040$), while the PLY

group had a positive relationship at the same sites (r= 0.581, p= 0.039). The PLY group also had a positive relationship between the percent change in whole body lean body mass and the percent change in hip bone mineral density (PLY: r= 0.565, p= 0.044; RT: r= 0.285, p= 0.229). The positive relationships between changes in lean body mass and changes in bone mineral density in the PLY group suggests that even small, non-significant changes in lean body mass may increase the ground reaction force during plyometric exercise. Thus, lean body mass changes may increase ground reaction forces, thereby potentially increasing or maintaining bone mass.

Also, the PLY group non-significantly increased the average vertical jump height, and there was a significant positive relationship between the percent change in vertical jump height and the percent change in hip bone mineral density (r= 0.756, p= 0.006). Thus, higher jumps may induce stronger ground reaction forces, and thereby, generate stronger impact forces when landing.

The data for the relationships between percent change in lean body mass and percent change in bone mineral density seem to indicate that increases in lean body mass may be a mechanism to increase the impact force while jumping.

For the average population, upper body plyometric exercises are not feasible. Thus, changes in upper body bone mineral density can only be attributed to muscle contractile forces and lean body mass. Since ground reaction forces will not have an effect on the change in bone mineral density, and the bone mineral density of the arm is purely dependent on lean body mass and

muscle contractile forces, we expected to see a similar positive relationship for both RT and PLY. There was a significant positive relationship between the percent change in left arm bone mineral density and left arm lean body mass in the RT group (RT: r= 0.577, p= 0.047; PLY: r= 0.189, p= 0.300). This finding seems to indicate that increases in left arm lean body mass may theoretically increase the muscle contraction onto the bone, and therefore, increase bone mineral density.

Hormonal Changes

After a year of either plyometric or resistance training exercise, the resting concentrations of SHBG, testosterone, free testosterone, and estradiol did not change. However, many studies have indicated that these hormones increase during an exercise session, and then decrease back to baseline values shortly after exercise has ceased [104]. We hypothesized that these hormones would gradually and chronically increase throughout the intervention, and then have a chronic, positive effect on bone mass; however, that was not the case.

Rogers et al. [32] looked at the acute response of testosterone to the same resistance training and plyometric protocol used in this study. They measured testosterone concentrations at baseline, immediately following exercise, and at 15 minutes, 30 minutes, 1 hour, 2 hours, and 24 hours after baseline to determine testosterone changes [32]. In both the resistance training and plyometric exercise bouts, they found testosterone concentrations to be

significantly increased 24 hours later [32]. It would be interesting to see how long the testosterone concentrations remain elevated.

As one ages, the concentration of SHBG is known to increase [20, 72]; however, both exercise groups saw non-significant decreases in SHBG concentrations (**Table 8**), which may benefit bone mass. Since proportions of testosterone and estradiol both bind to SHBG, thereby occupying their active sites, a lower concentrations of SHBG would suggest that there would be an increase in the concentration of bioavailable testosterone and estradiol. However, while we did not measure free estradiol, there was no significant change in the concentration of free testosterone.

We expected there to be positive, significant relationships between the percent changes in the sex hormones measured and the percent changes in the bone mineral density sites measured. However, there were only a few significant relationships, and they were all negative. Both the RT and PLY groups had negative significant relationships between the percent change in total testosterone and the percent change in hip bone mineral density (PLY: r= -0.643, p= 0.043; RT: r= -0.614, p= 0.039). The RT group also had a negative significant relationship between the percent change in estradiol and the percent change in hip bone mineral density (RT: r= -0.735, p= 0.030; PLY: r= -0.440, p= 0.118). Perhaps with more subjects, there would be stronger relationships between the hormones and the bone mineral density sites measured.

There were no other significant relationships between the sex hormones and bone density. Thus, it seems that bone mineral density in osteopenic men is not dependent on increases in testosterone, free testosterone, or estradiol. Similarly, the current literature states that estrogen administration reduces fracture risk in post-menopausal women, but it does not reduce fracture risk in women with established osteoporosis [89]. This seems to suggest that hormonal interventions are beneficial in preventing bone loss but have little effect in increasing bone mass.

Further research may monitor sex hormone changes throughout resistance training and plyometric workouts to see: 1) if the concentrations increase during exercise, 2) if the increased exercise-induced concentrations increase over time, and 3) if there is a correlation between the exercise-induced increases in SHBG, testosterone, free testosterone, and/or estradiol and bone mineral density. It is currently thought that the concentrations of hormones change throughout an exercise bout; thus, it would be interesting to see if the exercise-induced changes in hormone concentrations were related to changes in bone mineral density sites.

Strengths

One of the main strengths of this study was that each exercise session was supervised by trained lab personnel for completion of the session, safety, and to ensure proper form was being used. In addition, each session was documented, and ratings of perceived pain (scale of 0-100, with 0 being no pain

at all, and 100 is the most intense pain imaginable) were recorded for each lift or set of jumps. On average, the PLY and RT groups rated their pain as 4 and 10, respectively. Thus, the exercises were appropriate for this population and did not have a significant risk of injury or harm.

The bone remodeling process can take up to 200 days [25]; thus, the 1-year duration of the present study ensures that each subject went through at least 1 cycle of bone turnover. Many previous studies failed to reach or exceed the length a full cycle of bone turnover [11, 24, 87, 88]. While the present study did exceed the length of a bone cycle, perhaps a longer study that captured several bone cycles would have obtained more definitive results. However, with a year-long study, we already had complications with subject retention and recruitment, and a longer study would have proved that much more difficult to achieve our sample size. This study also included calcium and vitamin D supplementation, which are vital nutrients in bone health [78]. Without adequate calcium and vitamin D, the subjects may not have the proper building blocks to strengthen bone.

Several of the previous studies used resistance machines [9, 10, 12, 88]. However, our RT program was specifically designed to load the axial skeleton, i.e. the hips and spine. All lifts were functional, standing, free-weight exercises, and trained lab personal was always present to spot. Maddalozzo et al. [9] split their subjects (male and female) into 2 groups, a high-intensity and a moderate-intensity resistance training group. The program for both groups activated all

major muscle groups. However, the moderate-intensity groups used seated, machine-based exercises at 40-60% of 1RM, while the high-intensity group had a standing free-weight program at 70-90% of 1RM [9]. They found the men in the high-intensity group had a 1.9% increase in lumbar spine bone mineral density, while there was no change in lumbar spine for the women in the high-intensity group nor either gender in the moderate-intensity group [9]. Both the men and women in the high-intensity group had increases in trochanteric bone mineral density (1.3% and 2.0%, respectively) [9]. Thus, it is unclear whether the free-weights or the increase in intensity induced the changes in bone mineral density, but for purposes of this study, we thought it would induce greater changes in bone mass if the resistance force was directly loading the spine and hip bones.

Another strength of the present study was that the PLY program included jumps that were multi-directional. Research has shown that high-impact sports that stress the bone in multiple directions, such as volleyball and gymnastics, are associated with a higher bone mineral density than sports consisting of only one direction of movement (e.g. running and cycling) [2]. Bones may become desensitized to the jumps without regular rest intervals [2]. Thus, our subjects took a 10 second rest period between each jump, and a 30 second rest period between series of 5 bounding jumps.

The periodized nature of both the RT and PLY program strengthened this study as well. Research has shown that bone adapts to the current loading magnitude [2]. Thus, the RT and PLY programs were designed to go through a

7-week mesocycle, in which 2 weeks are light, 2 are moderate, 2 are heavy, and then 1 rest week. This ensures that the muscles and bones do not stop adapting to the current stimulus. In addition, the PLY program consisted of (at most) 100 jumps, and the RT program was only 6 exercises. These programs were designed so they could easily fit into one's current exercise regime.

Limitations

Along with all the strengths of the current study, there were several limitations, including the sample size, an insufficient energy intake to maximize the increase in lean body mass in the RT group, and the lack of a control group. This study only had 20 subjects. The current study is part of a larger study that intends to achieve a sample size of 40 or more. Hopefully, with 40 subjects, the results will be stronger.

While we did collect five 7-day diet logs and physical activity logs from the subjects during the study, self-reporting is not always the more accurate representation of the daily diet. Due to many of our subjects being active in endurance sports, it is possible that some subjects may have been in a caloric deficit, which induces bone loss, and may also influence the results.

A control group may also have strengthened the results of the current study. As previously noted, even with a high calcium diet and bone-loading physical activity, it is difficult to increase bone mass past the young adult years. It is thought that osteopenic men not participating in resistance training or plyometric activity on a regular basis are, in fact, decreasing their bone mass

[99]; thus, it was unethical to put osteopenic men in a control group knowing their bone mass would suffer. While we did try to recruit men that were eligible for the study but chose not to participate as our controls, we were not able to recruit enough for statistical power. Thus, the maintenance of bone mass in this population may have been a significant finding had there been a control group for comparison.

FURTHER DIRETIONS

Since the present study established a periodized resistance training program and a periodized plyometric exercise program that increased or maintained bone mineral density in active, osteopenic, adult males, future studies should establish the lowest threshold of resistance training or plyometric exercise needed to positively impact bone. It would also be interesting to see future studies examine the effectiveness of an exercise-based treatment versus a drug-based treatment on the maintenance or acquisition of bone mass.

One of the aims of the present study was to determine the effect lean body mass may have on bone mineral density acquisition. However, in both the RT and PLY groups, there were very small changes in lean body mass. While this study was designed to increase bone mineral density, it would be interesting to design an exercise program that aimed to increase lean body mass, and then see if there were any relationships between bone mineral density and lean body mass.

As indicated, the sex hormone concentrations did not change in either group. The lack of change may indicate that these hormones are not significant factors in bone mineral density acquisition in osteopenic men. However, it would be interesting to monitor the sex hormone changes throughout a resistance training or plyometric workout to see if the concentrations changed during the workout and immediately post-workout. If there were exercise-induced changes in hormone concentrations, it would be interesting to see if the exercise-induced changes in the sex hormones were correlated to changes in bone mineral density.

While the present study established an exercise prescription for management of osteopenia, further research is needed to determine the factors that contributed to that increase.

CONCLUSION

The results of the present study indicate that exercise may be an alternative treatment to anti-resorptive osteoporotic drug therapy for osteopenic males. The sites commonly measured for the detection of low bone mass are the hip and the lumbar spine. The aims of this study did not cover the lumbar spine; however, we did find that the hip bone mineral density significantly increased in the RT group, and remained unchanged in the PLY group. Thus, the exercise protocols in this study have clinical significance and may reduce fracture risk in this population.

This study suggests that inclusion of either a periodized resistance training program that aims at loading the hip and spine, or a multi-directional plyometric program into one's current exercise regime will aid in maintaining and may even increase bone mineral density in active, osteopenic, adult males. The plyometric group increased bone mineral density of the whole body and left leg. The resistance training group increased bone mineral density of the whole body, left leg, and hip. It is important to note that neither group had a decrease in bone mineral density at any site. The present study also indicates that testosterone, free testosterone, and estradiol may not be significant hormonal players in bone mass acquisition in active, osteopenic, adult males. Thus, 2-3 bouts a week of plyometric or resistance training activity may be enough to stimulate osteogenesis and can be beneficial for men with low bone mineral density.

REFERENCES

1. Bakhireva, L.N., et al., *Modifiable predictors of bone loss in older men: a prospective study.* Am J Prev Med, 2004. **26**(5): p. 436-42.

2. Bailey, C.A. and K. Brooke-Wavell, *Exercise for optimising peak bone mass in women.* Proc Nutr Soc, 2008. **67**(1): p. 9-18.

3. Shibata, Y., et al., *Effects of physical training on bone mineral density and bone metabolism.* J Physiol Anthropol Appl Human Sci, 2003. **22**(4): p. 203-8.

4. *Low bone density or "osteopenia"--what does it mean?* 2009 [cited 2011 January 13, 2011]; Available from: http://www.nof.org/aboutosteoporosis/detectingosteoporosis/osteopoenia.

5. Rector, R.S., et al., *Participation in road cycling vs running is associated with lower bone mineral density in men.* Metabolism, 2008. **57**(2): p. 226-32.

6. Rector, R.S., et al., *Lean body mass and weight-bearing activity in the prediction of bone mineral density in physically active men.* J Strength Cond Res, 2009. **23**(2): p. 427-35.

7. Ongphiphadhanakul, B., et al., *Serum testosterone and its relation to bone mineral density and body composition in normal males.* Clin Endocrinol (Oxf), 1995. **43**(6): p. 727-33.

8. Layne, J.E. and M.E. Nelson, *The effects of progressive resistance training on bone density: a review.* Med Sci Sports Exerc, 1999. **31**(1): p. 25-30.

9. Maddalozzo, G.F. and C.M. Snow, *High intensity resistance training: effects on bone in older men and women.* Calcif Tissue Int, 2000. **66**(6): p. 399-404.

10. Ryan, A.S., et al., *Regional bone mineral density after resistive training in young and older men and women.* Scand J Med Sci Sports, 2004. **14**(1): p. 16-23.

11. Guadalupe-Grau, A., et al., *Strength training combined with plyometric jumps in adults: sex differences in fat-bone axis adaptations.* J Appl Physiol, 2009. **106**(4): p. 1100-11.

12. Karinkanta, S., et al., *A multi-component exercise regimen to prevent functional decline and bone fragility in home-dwelling elderly women: randomized, controlled trial.* Osteoporos Int, 2007. **18**(4): p. 453-62.

13. Creighton, D.L., et al., *Weight-bearing exercise and markers of bone turnover in female athletes.* J Appl Physiol, 2001. **90**(2): p. 565-70.

14. Duncan, C.S., et al., *Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength.* Med Sci Sports Exerc, 2002. **34**(2): p. 286-94.

15. Madsen, K.L., W.C. Adams, and M.D. Van Loan, *Effects of physical activity, body weight and composition, and muscular strength on bone density in young women.* Med Sci Sports Exerc, 1998. **30**(1): p. 114-20.

16. Marin, R.V., et al., *Association between lean mass and handgrip strength with bone mineral density in physically active postmenopausal women.* J Clin Densitom, 2010. **13**(1): p. 96-101.

17. Soot, T., et al., *Relationship between leg bone mineral values and muscle strength in women with different physical activity.* J Bone Miner Metab, 2005. **23**(5): p. 401-6.

18. Li, S., et al., *Relationship between soft tissue body composition and bone mass in perimenopausal women.* Maturitas, 2004. **47**(2): p. 99-105.

19. Winters, K.M. and C.M. Snow, *Body composition predicts bone mineral density and balance in premenopausal women.* J Womens Health Gend Based Med, 2000. **9**(8): p. 865-72.

20. Tuck, S.P., et al., *Sex steroids and bone turnover markers in men with symptomatic vertebral fractures.* Bone, 2008. **43**(6): p. 999-1005.

21. Melton, L.J., 3rd, et al., *Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation.* J Bone Miner Res, 1997. **12**(1): p. 16-23.

22. Watts, N.B., et al., *Osteoporosis in men: an endocrine society clinical practice guideline.* J Clin Endocrinol Metab, 2012. **97**(6): p. 1802-22.

23. Ehrnborg, C., et al., *The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test.* J Clin Endocrinol Metab, 2003. **88**(1): p. 394-401.

24. Fujimura, R., et al., *Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism.* J Bone Miner Res, 1997. **12**(4): p. 656-62.

25. Allain, T.J. and A.M. McGregor, *Thyroid hormones and bone.* J Endocrinol, 1993. **139**(1): p. 9-18.

26. Almstedt, H.C., et al., *Changes in bone mineral density in response to 24 weeks of resistance training in college-age men and women.* J Strength Cond Res, 2011. **25**(4): p. 1098-103.

27. Frost, H.M., *Why do marathon runners have less bone than weight lifters? A vital-biomechanical view and explanation.* Bone, 1997. **20**(3): p. 183-9.

28. Nichols, J.F., J.E. Palmer, and S.S. Levy, *Low bone mineral density in highly trained male master cyclists.* Osteoporos Int, 2003. **14**(8): p. 644-9.

29. Warner, S.E., J.M. Shaw, and G.P. Dalsky, *Bone mineral density of competitive male mountain and road cyclists.* Bone, 2002. **30**(1): p. 281-6.

30. Guadalupe-Grau, A., et al., *Exercise and bone mass in adults.* Sports Med, 2009. **39**(6): p. 439-68.

31. Chatzinikolaou, A., et al., *Time course of changes in performance and inflammatory responses after acute plyometric exercise.* J Strength Cond Res, 2010. **24**(5): p. 1389-98.

32. Rogers, R.S., et al., *Acute response of serum markers of bone turnover to a single-bout of resistance-training or plyometrics.* J Appl Physiol, 2011.

33. Nagasawa, S., et al., *Effects of low-repetition jump exercise on osteogenic response in rats.* J Bone Miner Metab, 2008. **26**(3): p. 226-30.

34. Rantalainen, T., et al., *Three-month bilateral hopping intervention is ineffective in initiating bone biomarker response in healthy elderly men.* Eur J Appl Physiol, 2011. **111**(9): p. 2155-62.

35. Witzke, K.A. and C.M. Snow, *Lean body mass and leg power best predict bone mineral density in adolescent girls.* Med Sci Sports Exerc, 1999. **31**(11): p. 1558-63.

36. Bilanin, J.E., M.S. Blanchard, and E. Russek-Cohen, *Lower vertebral bone density in male long distance runners.* Med Sci Sports Exerc, 1989. **21**(1): p. 66-70.

37. Colletti, L.A., et al., *The effects of muscle-building exercise on bone mineral density of the radius, spine, and hip in young men.* Calcif Tissue Int, 1989. **45**(1): p. 12-4.

38. Honda, A., et al., *Bones benefits gained by jump training are preserved after detraining in young and adult rats.* J Appl Physiol, 2008. **105**(3): p. 849-53.

39. Swift, J.M., et al., *Increased training loads do not magnify cancellous bone gains with rodent jump resistance exercise.* J Appl Physiol, 2010. **109**(6): p. 1600-7.

40. Umemura, Y., et al., *Five jumps per day increase bone mass and breaking force in rats.* J Bone Miner Res, 1997. **12**(9): p. 1480-5.

41. Robling, A.G., *Is bone's response to mechanical signals dominated by muscle forces?* Med Sci Sports Exerc, 2009. **41**(11): p. 2044-9.

42. Frost, H.M., *Bone's mechanostat: a 2003 update.* Anat Rec A Discov Mol Cell Evol Biol, 2003. **275**(2): p. 1081-101.

43. Weeks, B.K. and B.R. Beck, *The BPAQ: a bone-specific physical activity assessment instrument.* Osteoporos Int, 2008. **19**(11): p. 1567-77.

44. Stewart, A.D. and J. Hannan, *Total and regional bone density in male runners, cyclists, and controls.* Med Sci Sports Exerc, 2000. **32**(8): p. 1373-7.

45. Heinonen, A., et al., *Bone mineral density of female athletes in different sports.* Bone Miner, 1993. **23**(1): p. 1-14.

46. Dook, J.E., et al., *Exercise and bone mineral density in mature female athletes.* Med Sci Sports Exerc, 1997. **29**(3): p. 291-6.

47. Umemura, Y., et al., *Effects of jump training on bone hypertrophy in young and old rats.* Int J Sports Med, 1995. **16**(6): p. 364-7.

48. Vingren, J.L., et al., *Testosterone physiology in resistance exercise and training: the up-stream regulatory elements.* Sports Med, 2010. **40**(12): p. 1037-53.

49. Cardinale, M. and M.H. Stone, *Is testosterone influencing explosive performance?* J Strength Cond Res, 2006. **20**(1): p. 103-7.

50. Bardin, C.W., *The anabolic action of testosterone.* N Engl J Med, 1996. **335**(1): p. 52-3.

51. Kudlac, J., et al., *Impact of detraining on bone loss in former collegiate female gymnasts.* Calcif Tissue Int, 2004. **75**(6): p. 482-7.

52. Kraemer, W.J. and N.A. Ratamess, *Hormonal responses and adaptations to resistance exercise and training.* Sports Med, 2005. **35**(4): p. 339-61.

53. Izquierdo, M., et al., *Cytokine and hormone responses to resistance training.* Eur J Appl Physiol, 2009. **107**(4): p. 397-409.

54. Cadore, E.L., et al., *Hormonal responses to resistance exercise in long-term trained and untrained middle-aged men.* J Strength Cond Res, 2008. **22**(5): p. 1617-24.

55. Bebo, B.F., Jr., et al., *Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells.* J Immunol, 1999. **162**(1): p. 35-40.

56. Shea, M.K., et al., *Vitamin K, circulating cytokines, and bone mineral density in older men and women.* Am J Clin Nutr, 2008. **88**(2): p. 356-63.

57. Cauley, J.A., et al., *Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study.* J Bone Miner Res, 2007. **22**(7): p. 1088-95.

58. Gatti, R. and E.F. De Palo, *An update: salivary hormones and physical exercise.* Scand J Med Sci Sports, 2011. **21**(2): p. 157-69.

59. Tremblay, M.S., J.L. Copeland, and W. Van Helder, *Effect of training status and exercise mode on endogenous steroid hormones in men.* J Appl Physiol, 2004. **96**(2): p. 531-9.

60. Tremblay, M.S., J.L. Copeland, and W. Van Helder, *Influence of exercise duration on post-exercise steroid hormone responses in trained males.* Eur J Appl Physiol, 2005. **94**(5-6): p. 505-13.

61. Cadore, E., et al., *Correlations between serum and salivary hormonal concentrations in response to resistance exercise.* J Sports Sci, 2008. **26**(10): p. 1067-72.

62. Bosco, C., et al., *Hormonal responses in strenuous jumping effort.* Jpn J Physiol, 1996. **46**(1): p. 93-8.

63. Michael, H., et al., *Estrogen and testosterone use different cellular pathways to inhibit osteoclastogenesis and bone resorption.* J Bone Miner Res, 2005. **20**(12): p. 2224-32.

64. Orwoll, E.S., J.P. Bilezikian, and D. Vanderschueren, *Osteoporosis in men : the effects of gender on skeletal health*. 2nd ed. 2010, Amsterdam ; Boston: Elsevier Academic Press. xv, 741, 5 p. of plates.

65. Khosla, S., et al., *Relationship of volumetric BMD and structural parameters at different skeletal sites to sex steroid levels in men.* J Bone Miner Res, 2005. **20**(5): p. 730-40.

66. Ackerman, K.E., et al., *Estradiol levels predict bone mineral density in male collegiate athletes: a pilot study.* Clin Endocrinol (Oxf), 2012. **76**(3): p. 339-45.

67. Greendale, G.A., S. Edelstein, and E. Barrett-Connor, *Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study.* J Bone Miner Res, 1997. **12**(11): p. 1833-43.

68. Center, J.R., et al., *Hormonal and biochemical parameters in the determination of osteoporosis in elderly men.* J Clin Endocrinol Metab, 1999. **84**(10): p. 3626-35.

69. Gu, G., et al., *Estrogen protects primary osteocytes against glucocorticoid-induced apoptosis.* Apoptosis, 2005. **10**(3): p. 583-95.

70. Khosla, S., et al., *Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men.* J Clin Endocrinol Metab, 2001. **86**(8): p. 3555-61.

71. Araujo, A.B., et al., *Correlations between serum testosterone, estradiol, and sex hormone-binding globulin and bone mineral density in a diverse sample of men.* J Clin Endocrinol Metab, 2008. **93**(6): p. 2135-41.

72. Szulc, P., et al., *Bioavailable estradiol may be an important determinant of osteoporosis in men: the MINOS study.* J Clin Endocrinol Metab, 2001. **86**(1): p. 192-9.

73. Consitt, L.A., J.L. Copeland, and M.S. Tremblay, *Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women.* Sports Med, 2002. **32**(1): p. 1-22.

74. Nakamura, Y., et al., *Hormonal responses to resistance exercise during different menstrual cycle states.* Med Sci Sports Exerc, 2011. **43**(6): p. 967-73.

75. Brown, G.A., et al., *Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men.* Int J Sport Nutr Exerc Metab, 2000. **10**(3): p. 340-59.

76. Nackaerts, O., et al., *Is self-reported alcohol consumption associated with osteoporotic mandibular bone loss in women?* Eur J Oral Sci, 2009. **117**(1): p. 7-12.

77. Kriska, A.M., et al., *The assessment of historical physical activity and its relation to adult bone parameters.* Am J Epidemiol, 1988. **127**(5): p. 1053-63.

78. Cranney, A., et al., *Effectiveness and safety of vitamin D in relation to bone health.* Evid Rep Technol Assess (Full Rep), 2007(158): p. 1-235.

79. Willis, K.S., N.J. Peterson, and D.E. Larson-Meyer, *Should we be concerned about the vitamin D status of athletes?* Int J Sport Nutr Exerc Metab, 2008. **18**(2): p. 204-24.

80. Ly, L.P. and D.J. Handelsman, *Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays.* Eur J Endocrinol, 2005. **152**(3): p. 471-8.

81. Clinic, M. *Testosterone, Total, Bioavailable, and Free, Serum*. Test ID: TTFB 2012.

82. Bassey, E.J., et al., *Pre- and postmenopausal women have different bone mineral density responses to the same high-impact exercise.* J Bone Miner Res, 1998. **13**(12): p. 1805-13.

83. Engelke, K., et al., *Exercise maintains bone density at spine and hip EFOPS: a 3-year longitudinal study in early postmenopausal women.* Osteoporos Int, 2006. **17**(1): p. 133-42.

84. Kerr, D., et al., *Exercise effects on bone mass in postmenopausal women are site-specific and load-dependent.* J Bone Miner Res, 1996. **11**(2): p. 218-25.

85. Kohrt, W.M., A.A. Ehsani, and S.J. Birge, Jr., *Effects of exercise involving predominantly either joint-reaction or ground-reaction forces on bone*

mineral density in older women. J Bone Miner Res, 1997. **12**(8): p. 1253-61.

86. Wallace, B.A. and R.G. Cumming, *Systematic review of randomized trials of the effect of exercise on bone mass in pre- and postmenopausal women.* Calcif Tissue Int, 2000. **67**(1): p. 10-8.

87. Menkes, A., et al., *Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men.* J Appl Physiol, 1993. **74**(5): p. 2478-84.

88. Yarasheski, K.E., J.A. Campbell, and W.M. Kohrt, *Effect of resistance exercise and growth hormone on bone density in older men.* Clin Endocrinol (Oxf), 1997. **47**(2): p. 223-9.

89. Levis, S. and G. Theodore, *Summary of AHRQ's comparative effectiveness review of treatment to prevent fractures in men and women with low bone density or osteoporosis: update of the 2007 report.* J Manag Care Pharm, 2012. **18**(4 Suppl B): p. S1-15; discussion S13.

90. Pasco, J.A., et al., *Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study.* J Bone Miner Res, 2004. **19**(1): p. 19-24.

91. Reid, I.R., et al., *Intravenous zoledronic acid in postmenopausal women with low bone mineral density.* N Engl J Med, 2002. **346**(9): p. 653-61.

92. Liberman, U.A., et al., *Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group.* N Engl J Med, 1995. **333**(22): p. 1437-43.

93. Weinstein, R.S., P.K. Roberson, and S.C. Manolagas, *Giant osteoclast formation and long-term oral bisphosphonate therapy.* N Engl J Med, 2009. **360**(1): p. 53-62.

94. Shane, E., *Evolving data about subtrochanteric fractures and bisphosphonates.* N Engl J Med, 2010. **362**(19): p. 1825-7.

95. Wishart, J.M., et al., *Effect of age on bone density and bone turnover in men.* Clin Endocrinol (Oxf), 1995. **42**(2): p. 141-6.

96. M.O. Widzer, R.S.R., P.S. Hinton, *Three year follow of bone density in male athletes with osteopenia*, in *Central States Chapter of the American College of Sports Medicine*. 2009: Columbia, MO.

97. Petit, M.A., et al., *A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study.* J Bone Miner Res, 2002. **17**(3): p. 363-72.

98. Adami, S., et al., *Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women.* J Bone Miner Res, 1999. **14**(1): p. 120-4.

99. Riggs, B.L., S. Khosla, and L.J. Melton, 3rd, *A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men.* J Bone Miner Res, 1998. **13**(5): p. 763-73.

100. Knoke, J.D. and E. Barrett-Connor, *Weight loss: a determinant of hip bone loss in older men and women. The Rancho Bernardo Study.* Am J Epidemiol, 2003. **158**(12): p. 1132-8.

101. Bell, G.J., et al., *Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans.* Eur J Appl Physiol, 2000. **81**(5): p. 418-27.

102. Bouchla, A., et al., *The addition of strength training to aerobic interval training: effects on muscle strength and body composition in CHF patients.* J Cardiopulm Rehabil Prev, 2011. **31**(1): p. 47-51.

103. Burr, D.B., *Muscle strength, bone mass, and age-related bone loss.* J Bone Miner Res, 1997. **12**(10): p. 1547-51.

104. Zmuda, J.M., P.D. Thompson, and S.J. Winters, *Exercise increases serum testosterone and sex hormone-binding globulin levels in older men.* Metabolism, 1996. **45**(8): p. 935-9.

EXTENDED LITERATURE REVIEW

I. Bone Physiology

Bone is a metabolically active tissue that is constantly undergoing turnover [1]. Bone is composed of 70% hydroxyapatite crystals, 22% proteins, and 8% water; it is the quality and arrangement of these constituents that determines the strength of the bone [2]. The proportion of each depends on the particular type of bone, and the two types of bone are trabecular and cortical bone [3]. Bone types vary from long and short bones, such as the femur in the leg and the carpals in the palm of the hand, respectively, to flat bones, such as the scapula, or the shoulder blade. There are also irregular bones, such as the vertebra, and sesamoid bones, like the patella, i.e. the knee cap.

There are 213 bones in the adult human skeleton [4]. Each bone is formed through a process called modeling, and it is constantly renewed and repaired through a process termed remodeling [4]. The functions of bone vary depending on the type and location of the bone but include structural support, locomotion, protection of vital organs, and maintenance of mineral homeostasis [4].

A long bone (**Figure 17**) consists of a long hollow shaft, or diaphysis [4]. The diaphysis flares at the ends to form the metaphysis, which is located just below the growth plate, and the epiphysis, which is just above the growth plate [4]. The diaphysis is composed of cortical bone, while the metaphysis and epiphysis are made of trabecular bone, with a shell of cortical bone surrounding

the exterior [4]. The periosteum is the outer fibrous sheath that covers all bone tissue except at the joints, which are lined with articular cartilage [4]. The blood vessels, nerve endings, and bone remodeling cells are all housed in the periosteum [4]. The endosteum is a membranous sheath that lines the inner surface of the bone and is in direct contact with the bone marrow [4]. While it also contains blood vessels and bone remodeling cells, the endosteum also lines the blood vessel canals that run through the bone [4].

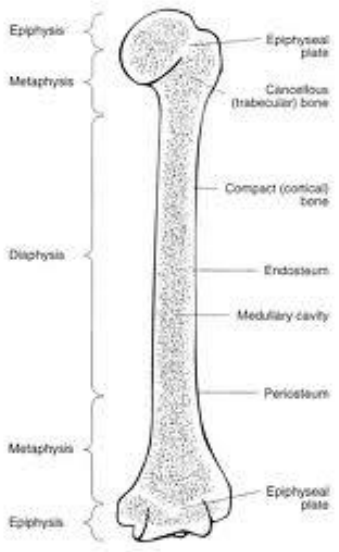


FIGURE 17. Adapted from Copstead et al. [5]. Depiction of a long bone. The long shaft is termed the diaphysis. On either end of the diaphysis, the shaft forms the metaphysis, which is just below the growth plate, and the epiphysis, which is just above the growth plate. The shaft is composed compact cortical bone, while the metaphysis and epiphysis contain trabecular bone interiorly and with a shell of cortical bone on the exterior.

As one ages, there is an associated increase in fracture risk that may be attributed to the decline in the mechanical competence of bone, which is related to the deterioration of the structural properties and the intrinsic material

properties of the bone [2]. Structural properties of bone include size, geometry, and the microstructural properties of the bone, including trabecular orientation and cortical porosity [2]. The intrinsic material properties of the bone are the bone mineral density, chemical composition, and the size of the hydroxyapatite crystals, which is the naturally occurring mineralized from of calcium [2]. Assessing one's bone mineral density is the current method of measuring mechanical competence of bones and is used to determine ones' fracture risk. The assessment of fracture risk is focused on sites that are prone to fractures and have a high proportion of trabecular bone, such as the lumbar spine, hip, and wrist [2].

The classification of bone as either trabecular or cortical is dependent on the porosity of the bone tissue, which is defined as the volume of the bone tissue that is occupied by non-mineralized tissue [6]. Trabecular bone has a porosity of 30-90%, while cortical bone has a porosity of 5-30% [6].

Trabecular Bone

Trabecular bone has been described as a "framework of delicate, intricate processes organized to support the bone marrow and to provide strength to the epiphyses and metaphyses" [7]. Trabecular bone, commonly known as "spongy" bone or cancellous bone, has a porous, lattice-like structure of interconnected trabecular plates [4, 8]. Trabecular bone typically occurs at the ends of long bones, in the vertebrae, and in flat bones [9]. It has a higher surface area and is highly vascularized, but is less dense, less stiff, and weaker than cortical bone

[8]. Since trabecular bone experiences the mechanical strains imposed on the skeleton, it has a higher rate of remodeling and is known to be more metabolically active than cortical bone [4, 10].

Trabecular bone only makes up 20% of the human skeleton, and it functions to distribute and dissipate the impact from mechanical loading, and it maintains the mechanical integrity of the bone, all while minimizing extra weight [8, 10]. In diseases such as osteoporosis, or low bone density, there is a reduction in the thickness, amount, and microarchitecture of trabecular bone [10].

It has been suggested that the risk of fracture is influenced not only by bone density, but by the microarchitecture of the bone as well [10]. Bone microarchitecture determines bone quality, strength, and structure. Differences in bone composition may be a large determinant in fracture risk, even for those with identical bone mineral densities [11]. As such, it has been suggested that trabecular bone is a major and independent determining factor of vertebral factors in men with osteoporosis [11].

Cortical Bone

Cortical bone, also known as compact bone, makes up 80% of the human skeleton, as it comprises the shaft of long bones and covers the surface of all bones [2, 4]. Cortical bone is stronger, harder, thicker, stiffer, and denser than trabecular bone [6, 9]. Cortical bone is primarily loaded during bending movements, and it is associated with a great amount of tensile strain [2]. Mineral content and bone density are determinants of the stiffness of cortical bone;

however, excessive mineralization will actually increase the fracture risk of the bone, as the bone becomes more brittle [2].

The size and distribution of the hydroxyapatite crystals determines the mechanical properties of cortical bone [2]. Collagen fibrils, other proteins, age, diet, drugs, and bone diseases are a few of the factors that affect mineral crystal size [2]. Young, healthy bone is composed of newly formed small crystals and mature larger crystals; the combination of both small and large crystals provides the bone with an ideal composition that resists fractures [2]. As one ages, the average crystal size increases, and the bone increasingly becomes more brittle [2]. The changes in hydroxyapatite crystal size are just one factor that leads to an increase in fracture risk as we age.

II. Bone Cells

The cells responsible for bone formation and bone resorption include chondrocytes, osteoblasts, and osteoclasts. The cells embedded in the bone matrix include the osteocytes. All of these bone cells are critical to maintain the structure, integrity, and function of bone.

Chondrocytes

Chondrocytes are the only cells found in cartilage [3]. According to the cartilage model, the skeleton is first formed with cartilage that is later replaced with an extracellular matrix that forms bone [3]. The cartilage model grows in length by continuous cell division of chondrocytes, and eventually is replaced by the extracellular matrix. Cartilage is important during the formation, growth, and

maintenance of long bones [3]. The cartilage model is the first step in endochondral ossification, which is the process in which most long and short bones develop and lengthen [3].

Osteoblasts

The main function of osteoblasts is to secrete a collage-based matrix that is then calcified to form bone [12]. These cells are known as the "bone builders," as they line the surface of the mineralized bone, synthesize new bone matrix, and regulate the mineralization and turnover of the bone matrix [3]. Once osteoblasts are mineralized, they become osteocytes and connect to one another through long processes called canaliculae [3].

Osteoclasts

Osteoclasts are responsible for the breakdown of bone [3]. They sense mechanical signals and convey the signals through the bone matrix [3]. When the osteoclasts receive a signal from the osteoblasts, they attach to the "ruffled border" of the bone and remove bone by producing an acid and transporting chloride out of the cell [3]. The acid dissolves the mineral, and once the mineral is removed, the osteoclasts release proteolytic enzymes that degrade the bone matrix [3]. During the process of bone matrix degradation, osteoblasts are signaled to begin new bone formation.

III. Endochondral ossification

Endochondral ossification (**Figure 18**) serves as a source of longitudinal bone growth and involves the replacement of the temporary cartilage model with permanent bone that is better suited to withstand the mechanical needs of an adult. This process of bone growth and development begins with the cartilage model in the fetus and is maintained through adolescence [13].

The cartilage model allows longitudinal bone growth. It is formed through the condensation of mesenchymal cells, which differentiate into chondrocytes and secrete a cartilage extracellular matrix component [13]. After the cartilage model is formed, the center of the cartilage is invaded by primary ossification centers (at the metaphysis), which gradually replace the cartilage with mineralized bone until skeletal maturity is reached [13]. After the primary centers of ossification arise in the center of the cartilage, the secondary centers of ossification (at the epiphysis) arise and begin to replace cartilage at the ends of the bone. The primary and secondary ossification centers are separated by the growth plate, and it is the articular-epiphyseal growth cartilage in the secondary centers of ossification that are responsible for shaping of the epiphysis and longitudinal bone growth [13].

After the chondrocytes closest to the centers of ossification go through proliferation, they transition in the “pre-hypertrophic” stage [13]. These chondrocytes then increase their size and at the same time begin to secrete the extracellular matrix, which is eventually mineralized through the deposition of hydroxyapatite, which is comprised of calcium and phosphate [13]. The combination of chondrocyte proliferation and secretion of an extracellular matrix is basis for longitudinal bone growth [13].

At this point, the hypertrophic chondrocytes then die, either through apoptosis [14] or by autophagic cell death [15]. The dead chondrocytes signal the breakdown of the cartilage matrix, thus allowing the entry of blood vessels,

osteoclasts, osteoblasts, and bone marrow cells [13]. The osteoclasts aid in removal of the cartilage matrix, while the osteoblasts use the remnants of the cartilage matrix as a base for bone deposition [13]. Since the osteoclasts remove cartilage matrix, which precedes the events that allow bone deposition on the cartilage remnants, and they allow entry of bone marrow cells, they are a necessary factor in establishing the primary ossification centers [13].

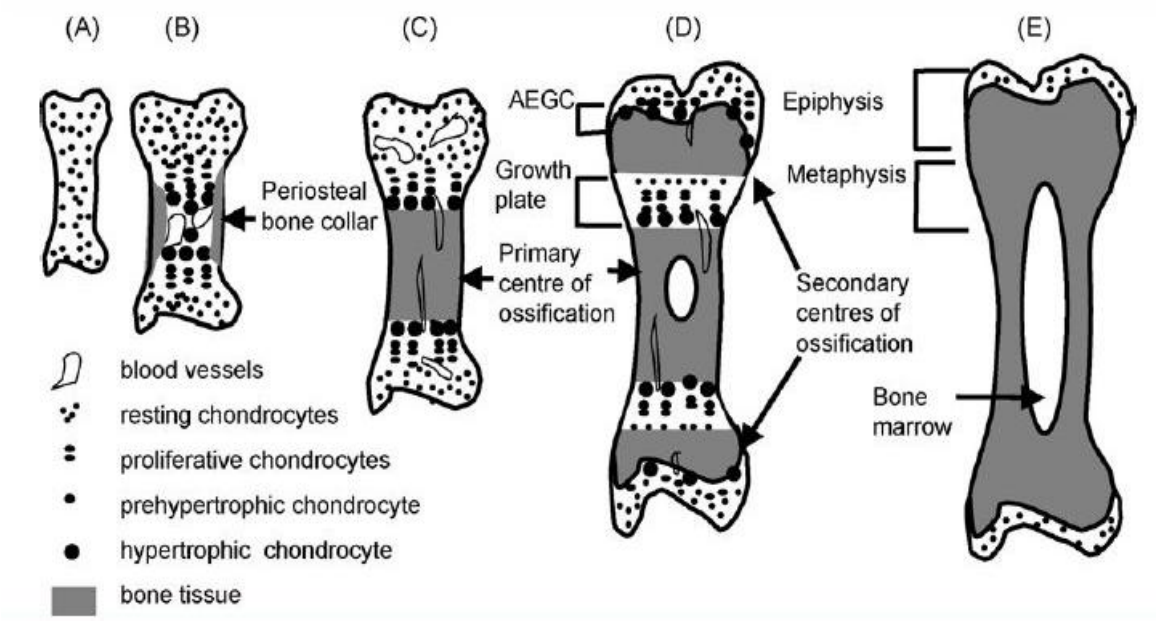


FIGURE 18. The cartilage model of bone development, adapted from Mackie et al [13]. (A) The embryonic cartilage model. (B) Initiation of the primary center of ossification, chondrocyte hypertrophy, and vasculation. (C) Primary center of ossification is established, and blood vessels are present in the remaining cartilage. (D) Secondary centers of ossification are formed, and are separated from the primary centers of ossification by the growth plate. The articular-epiphyseal growth cartilage (AEGC) is responsible for growth and shaping of the epiphysis and is located under the permanent articular cartilage. (E) The adult bone. The growth plate has disappeared, fusing the metaphysis and epiphysis together. The AEGC has been replaced by bone. The only remaining cartilage is the permanent articular cartilage at the end of each bone.

Skeletal maturity is reached when the centers of ossification replace the remaining cartilage with bone [13]. At this time, the metaphysis and epiphysis are fused together [13]; it has been postulated that the process of epiphyseal fusion occurs when the chondrocytes cease to proliferate [13]. In the adult bone, the only remaining cartilage is the permanent articular cartilage at the ends of long bones [13].

IV. Bone Remodeling

Bone modeling and remodeling are the processes by which bones are shaped or reshaped, respectively [4]. Bone tissue is shaped during growth and reshaped to respond to changes in mechanical loading during adulthood [4]. Bone modeling is distinguished from bone remodeling because bone formation is not tightly coupled with bone resorption [4]. This process occurs to a much lesser degree than bone remodeling in healthy adult bone, particularly trabecular bone [4]. Bone remodeling is a process that starts in utero and continues until death; it consists of the bone absorption phase, in which osteoclasts eat away small pits in the bone, and the bone formation phase, where osteoblasts create new bone [4].

To maintain skeletal homeostasis, the processes of bone absorption and bone formation must be tightly coupled. Many skeletal diseases, such as osteoporosis, are related to imbalances in the bone turnover cycle [12]. Remodeling of bone ensures mineral homeostasis, while maintaining the

structural integrity and bone strength by replacing old damaged bone with new bone [4, 16]. The rate of remodeling is dependent on the location of bone, age, and disease state. A typical bone cycle lasts approximately 120 days, or 4 months [1, 4]. The bone remodeling cycle can be thought of in five phases: activation, resorption, reversal, formation, and termination (**Figure 19**) [16].

Activation

The activation of bone remodeling can occur in response to microcracks, loss of mechanical loading, low blood calcium, or due to changes in hormones [17]. When the osteocytes, which are cells embedded in the bone matrix, sense any of these changes, they go through apoptosis [16, 17]. Osteocytes are thought to be the central regulators of the bone remodeling cycle, and one of their most important roles is to respond to changes and the mechanical stress placed on to bone [16, 17]. Osteocyte death or damage leads to increased osteoclastogenesis, which is the process by which osteoclasts are created [16].

Live osteocytes secrete transforming growth factor- β and osteoprotegerin, both of which inhibit resorption by osteoclasts and osteoclast production, thereby regulating the number of osteoclasts [16]. Osteocyte apoptosis seems to be a critical step in the activation of bone remodeling and osteoclast recruitment [16].

Resorption

The bone resorption phase lasts around three weeks [16, 17]. It is the process by which osteoclasts remove the damaged bone, thereby creating a pit in the bone matrix [16, 17]. Before the osteoclasts begin removing damaged bone, the

bone lining cells must pre-conditioning the bone surface, and the osteoclasts must be signaled to start differentiation. The process of osteoclast differentiation is thought to be controlled by close communication with either the osteocytes or the osteoblasts, i.e. the cells involved in bone formation [16]. During the bone resorption phase, the osteoclasts leave behind organic bone matrix and produce signals that lead to the reversal stage [16].

Reversal

After the osteoclasts finish resorbing, bone enters the reversal stage. During the reversal stage, bone lining cells (i.e. pre-osteoblasts) remove any remaining demineralized collagen matrix left in the resorbed pit before the osteoblasts precursors are recruited, thereby initiating bone formation [16]. Once the pit is cleaned, the bone lining cells differentiate into mature osteoblasts and initiate bone formation [18]. It is the bone lining cells that link the bone absorption process with the bone formation process [19].

Formation

Once the resorption holes are cleared, the bone lining cells either differentiate into osteoblasts or they are replaced with osteoblasts through a process that is controlled by osteoclast derived molecules (insulin growth factor-1, transforming growth factor- β, and cardiotrophin-1) [16]. The newly derived osteoblasts carry out bone formation in the newly resorbed bone pits until the resorbed area is rebuilt [16].

Just as bone resorption may be controlled by osteoblasts, bone formation is tightly regulated by osteoclasts [16]. Activation of osteoblasts is important for the bone formation phase of remodeling, and several molecules that induce bone formation have been shown to be secreted by osteoclasts or are released from the bone matrix during bone resorption [16]. Transforming growth factor- β and insulin growth factor-1 are both secreted by osteoclasts and released during bone resorption and may be important molecules in the process of osteoblast activation [16, 20, 21]. In addition, insulin growth factor-1 also controls parathyroid hormone, which regulates many steps of the bone remodeling cycles [16, 22]. It has also been suggested that osteoclasts produce cardiotrophin-1, which may lead to activation of bone formation by osteoblasts [23]. While bone formation is activated, bone absorption is inhibited, and the osteoclasts undergo calcium-induced apoptosis [17]. Bone formation takes about three months to complete [17].

Termination

Once bone formation is complete, bone lining cells line the bone surface, and the remodeling cycle is terminated [16]. Bone lining cells are inactive osteoblasts; they line the surface of all available bone [16]. Thus, once new bone is made, the bone remodeling cycle is terminated once the bone lining cells are present. This process is thought to be controlled through the direct contact of the newly generated osteocytes and the osteoblasts [16]. The osteocytes

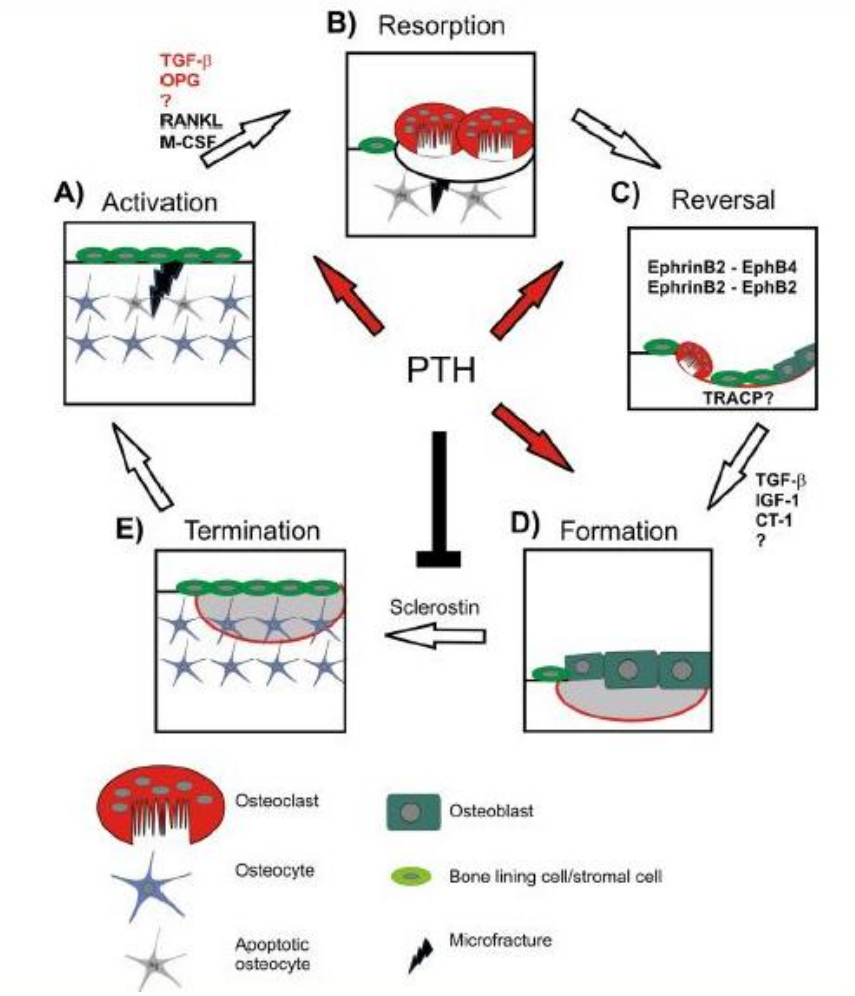


FIGURE 19. Adapted from Henriksen et al. [16]. Bone Remodeling Cycle: (A) Activation occurs when microcracks occur leading to osteocyte death and production of pro-osteoclastic signals, either through the osteocytes or through the bone lining cells. Production of transforming growth factor- β (TGF-β) and osteoprotegerin (OPG) regulate the number of osteoclasts. (B) The osteoclasts resorb bone, thereby removing the damaged bone matrix. (C) Bone lining cells clean the resorption pit. (D) The bone lining cells either differentiate into osteoblasts or are replaced by osteoblasts. (E) When bone formation is complete, the newly generated osteocytes secrete sclerostin which terminates the bone remodeling cycle. PTH regulates many of the steps.

secrete sclerostin, which is a ligand produced by the mature osteocytes in the bone matrix that prevents the activation of bone formation [16].

V. Low Bone Mass

Osteoporosis

Osteoporosis is the most common bone disorder, and according to the Consensus Development Conference in 1991, osteoporosis is characterized as "low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" [24]. According to the World Health Organization, osteoporosis is defined as a bone mineral density that is two-and-a-half standard deviations or more below the average value for a young adult (T-score < -2.5) [25]. The clinical significance of osteoporosis is the increased risk of a fragility fracture [26]. Consequently, osteoporosis is a leading cause of mortality, morbidity, and medical expense worldwide [24].

Currently, more than 75 million people in the United States, Europe, and Japan are diagnosed with osteoporosis, and over 4.5 million fractures occur annually in the United States and Europe [25]. The risk of a fracture is not the only risk that increases with osteoporosis, there is also a high prevalence of bedridden patients with serious, life-threating complications [25]. Due to the risk of osteoporotic-related morbidity, the prevention of low bone mass and associated fractures will improve health, quality of life, and independence in all those at risk for bone loss.

Many studies have found that low bone density at the lumbar spine, hip, and wrist significantly increases the risk of osteoporotic fracture [2]. Even though the most common osteoporotic fractures occur at the lumbar spine, hip, and wrist, the reduction of bone mineral density increases fracture risk at other sites as well [26]. Hip fracture is responsible for the majority of the mortality and morbidity associated with osteoporosis, and it is the leading cause of disability in the elderly [24]. It is well-established that the increased risk of death after a hip fracture is within the first three to six months after the injury [27]. Men consistently have a higher risk for complications after a hip fracture, including morbidity, mortality, loss of independence, and rate of institutionalization (**Figure 20**) [27, 28]. While hip fractures are associated with mortality, spine fractures are known to be a common source of pain, deformity, loss of height, and disability [27]. Research has indicated that the risk of a fragility fracture linearly increases as bone mineral density declines [29]. Fracture risk may increase 1.5 to 3-fold or greater for each standard deviation decrease in bone mineral density [26].

Osteoporosis is a disease that starts with the failure to achieve peak bone mass in youth, but usually does not manifest itself until the older adult/elderly years. The size, shape, and strength of adult bones is largely determined by the mechanical forces experienced during childhood and adolescence [30]. The mechanical loading of the skeleton during growth can significantly improve peak bone mass and offset the occurrence of bone loss and fracture later in life [30]. The accumulation of bone mass during adolescence, such that peak bone mass

is achieved, is a main factor in determining one's risk for osteoporosis or other bone issues as an adult [27]. Once peak bone mass is achieved, it is the maintenance of bone tissue during the adult life is of primary importance in reducing the risk of fractures, such that the rate and duration of bone loss is as negligible as possible.

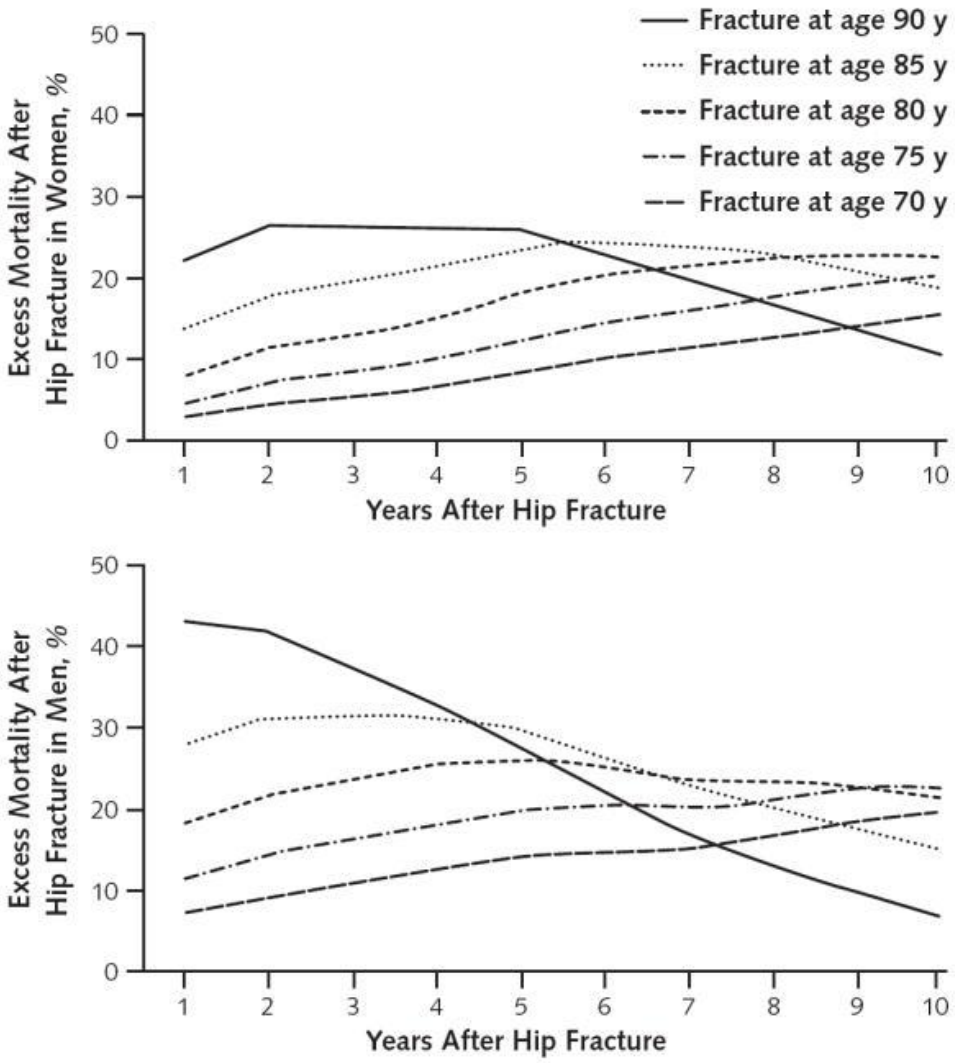


FIGURE 20. Adapted from Haentjens et al. [27]. Difference in excess mortality from all causes in men and women after a hip fracture when compared to a control group.

Osteopenia

For the most part, treatments for osteoporosis effectively maintain bone mineral density [26]. However, current osteoporosis treatments cannot increase low bone mass to normal values; therefore, maintaining or slowing the rate of deterioration is key in osteoporosis treatment [25]. Thus, recognition of low bone mass before it reaches osteoporosis is clinically relevant and may be the key in lowering fracture rates. Osteopenia is characterized as low bone mass, and is defined as a bone mineral density that is between one and two-and-a-half standard deviations below the young adult mean (T-score between -1 and -2.5) [25]. It is in this population that the prevention of bone loss is most clinically useful.

Bone Mineral Measurements

Bone geometry is known to adapt to changes in the mechanical environment, and many of these changes occur naturally with age [2]. Bone is maintained in areas that bear the largest loads while walking, and the greatest bone deterioration is seen in areas that are primarily loaded during falling [2]. Due to these structural changes in bone throughout the lifetime, it is important to assess fracture risk. The current standard of fracture risk assessment lies in bone mineral density measurement.

The most common sites measured for bone mineral density include the distal forearm, hip, and the lumbar spine due to the high risk of fracture among these sites [26]. Bone mineral density at one site is significantly correlated with

the bone mineral density at another site, and this correlation is stronger in the healthy, young population than in those with significant bone loss [26]. Individuals with low bone mass may have different rates of bone loss at different sites. Therefore, measurement of bone mass at all clinically significant sites increases the diagnosis and prevalence of osteoporosis and osteopenia.

When interpreting bone mineral density measurements, a single bone mineral density reading may not be very helpful in low risk populations [1]. Overtime, multiple readings may help assess whether that individual reached a low peak bone mass or is experiencing ongoing bone loss [1].

The most commonly used technique for bone mineral density assessment is the dual energy X-ray absorptiometry (DXA) [25]. Single-photon and dual-photon absorptiometry are also available as a bone mineral density assessment tool; however, they are noted to be less precise and accurate than the DXA [1]. As a method of determining bone mineral density, the ultrasound lacks radiation exposure and is economically reasonable; however, the results are not as reliable as that of the DXA [1]. Currently, CT scans and MRIs can be used to access the content of trabecular and cortical bone, which may be an important factor when assessing fracture risk [1]. While the DXA is a highly recognized as an accurate and precise tool to measure bone mineral density, it is not optimal for assessing fracture risk. In other words, fracture risk is increased with osteoporosis, but in those with normal bone mineral density, fracture risk is by no means negligible [25].

Microcracks

Microcracks (**Figure 21**) are short, linear splits in cortical bone tissue that range from 30-100 micrometers in length [2]. Cortical bone experiences microcracks after periods of prolonged loading and bone fatigue. Microcracks are associated with a significant degradation of bone stiffness [2]. Microcracks are a normal part of bone remodeling, and they occur through normal day-to-day activities. They tend to increase with age, and they are more prone to appear at weight-bearing sites [2]. Suppressing the growth of a crack seems to be more important to preventing fractures than suppressing the initiation of a crack [2]. As expected, there is a negative relationship between the number of microcracks in a bone and the stiffness and strength of a bone; however, it is unclear at which point the extent of the micro-damage will lead to an increase in fracture risk [2].

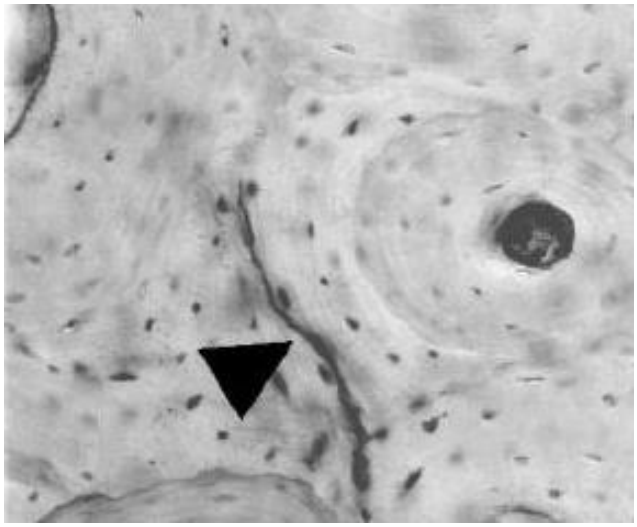


FIGURE 21. Adapted from Augat et al. [2]. A microcrack in human cortical bone; 100-fold magnification.

VI. Risk Factors for Low Bone Mass

The assessment of independent risk factors may enhance the information provided by the bone mineral density score [25]. Such risk factors include age, gender, ethnicity, family history, prior fragility fracture, low body mass index, smoking, excessive alcohol consumption, medications or diseases affecting bone, diet, and physical activity.

Low Peak Bone Mass

One of the main risk factors for osteoporosis or osteopenia is the achievement of a low peak bone mass [27]. Bone mass can continue to increase into the third decade of life, but peak accumulation of bone mass happens during adolescent years (**Figure 22**). There is a higher rate of bone turnover up until skeletal maturity, representing bone growth [31]. Bone formation and bone resorption markers both decrease in pre-menopausal women and in men between the ages of 20 and 50, thus suggesting bone homeostasis [31]. Around age 50 in men and after menopause in women, bone resorption is significantly increased, thereby reducing bone mass [31]. Thus, individuals who attain a high peak bone mass have more bone to lose bone mineral density declines to osteopenia or osteoporosis.

Factors such as low calcium intake, negative energy balance, and a lack of bone-loading physical activity during youth can predispose a person to a peak bone mass that is less than desirable. The lower one's peak bone mass, the closer they are to the osteopenic and osteoporotic ranges.

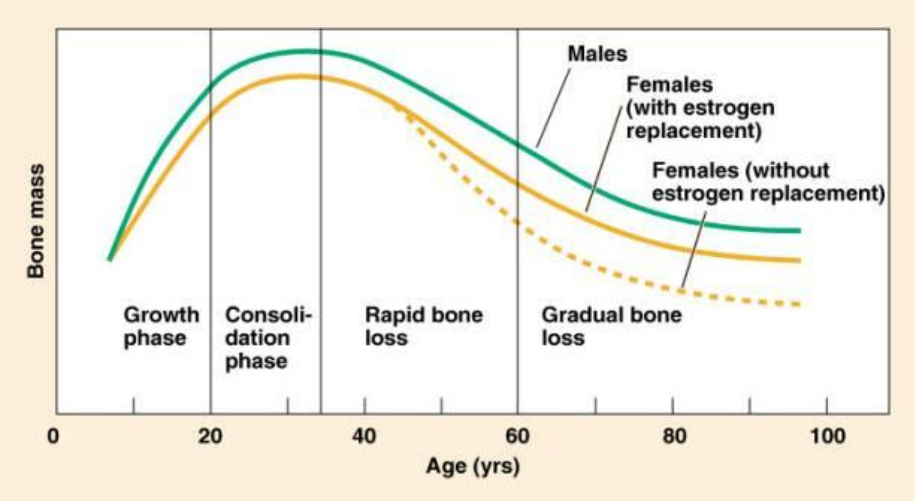


FIGURE 22. Adapted from Powers et al. [32]. Changes in bone mineral density throughout the lifespan in both men and women.

Age

Bone mineral density declines with advancing age, while fracture risk increases with age [1]. Due to the increase in the aging population and the rise in life expectancy in recent years, osteoporosis and fracture risks are expected to continue the upward trend [1]. After age 50, bone strength decreases 5-10% per decade [2]; thus, the prevalence of osteoporosis and osteoporotic-related fractures increases exponentially [26].

Through the aging process, the structure and intrinsic mechanical properties of bone change to adapt to the changes in mechanical stimuli [2]. The degradation of the intrinsic mechanical properties may be directly related to the bone remodeling cycle. As one ages, the remodeling cycle fails to replace all the bone that was previously removed, thereby increasing the porosity of cortical

bone [2]. As the porosity in the cortical tissue increases, the occurrence of microcracks increases as well; thus, the toughness of the bone is reduced [2].

Gender

Women are at a higher risk for osteoporosis than men [24]. Women that are thin and petite have the highest risk of low bone mass [24]. The peak bone mass of men is approximately 30% higher than that of women, and this is may be due to a longer pre-pubertal growth phase in men that elicits bones of a larger diameter [10]. Also, bone size is programed during pre-pubertal growth, which is two years longer in men than in women [10].

Even though more women are diagnosed with osteoporosis, men have the highest risk of mortality, morbidity, loss of independence, and rate of institutionalization [28]. It has been proposed that mortality associated hip fractures in men is at least twice as great as in women [11]. Moreover, vertebral deforming is also higher in men (29%) than in women (10%) [11, 33, 34]. In regards to trabecular and cortical bone, research has found that removal of trabecular bone is higher in women than in men [11], and men have a higher preservation of trabecular bone than women [35], and these changes are constant among the lifespan [11].

Family History

Genetics play a key role in skeletal fragility and osteoporosis [3, 24]. The current research estimates that as much as 50-80% of the variance in bone mineral density may arise from genetics, while the other 20-50% can be

attributed to lifestyle factors [3]. However, less research has been conducted on the heritability of a fracture; it has been suggested that 25-35% of the variance in fractures is genetically determined [3].

Genetics may influence skeletal growth and the amount of peak bone mass attained during adolescence [3]. Those that are genetically determined to achieve a low peak bone mass may be more susceptible to develop osteoporosis [3]. Along with the attainment of peak bone mass, genetic factors may play a role in the rate of bone loss [3]. However, research points to a greater genetic role in the acquisition of peak bone mass than to the rate of bone loss, which is influenced to a greater extent by environmental factors [36]. The greater effect of environmental factors on bone (i.e. nutrition, disease, medication, exercise, etc.) reduces the effect of heredity on bone. Another study that evaluated the genetics of bone mass established that the offspring of men with low bone mass have reduced bone size and reduced bone mineral density, despite having normal markers of bone remodeling [37]; thereby, pointing to a stronger genetic connection to bone growth than to age-related bone loss [3].

Ethnicity

Fracture rates differ greatly among different ethnicities. The risk of a hip fracture varies more than 10-fold between the European countries alone [25]. Due to a lower fracture risk and a shorter life expectancy, underdeveloped nations have the lowest absolute risk of hip fractures [25]. Those of Scandinavian, Caucasian, or Asian descent are among those with the highest

rates of hip fracture, while those from western Europe and southern Europe have a high and moderate risk, respectively [24, 25]. The Hispanic and African American races are among those with the highest bone mineral densities, as the fracture rate of an African American woman is one-third that of a Caucasian woman [1].

Endocrine

There are a variety of hormones and growth factors that affect bone, including growth hormone, insulin like growth factor-1, leptin, adiponectin, and parathyroid hormone [9]. While the literature has demonstrated a strong relationship between sex hormones and bone, the effects of testosterone, estradiol, and sex-hormone binding globulin will be discussed later.

Growth Hormone. Growth hormone has anabolic effects on both bone and skeletal muscle, and its secretion is known to decrease as one ages [38]. Both adult- and childhood-onset growth hormone deficiency are associated with reduced bone mineral densities and increased fracture rates [39]. Childhood-onset growth hormone deficiency is thought to be related to the failure to reach peak bone mass; while adult-onset growth hormone deficiency is associated with a bone mass reduction and is related to the failure to maintain bone mass [39]. There is a negative relationship between the age of onset of growth hormone deficiency and low bone mass, such that as the age of onset of growth hormone deficiency increases, the degree of low bone mass decreases [39].

The fracture rate is increased among men (4-fold) and women (2.5-fold) with growth hormone deficiency [40]. Growth hormone therapy may increase bone density and decrease fracture risk. Growth hormone therapy has been found to increase bone formation markers, bone resorption markers, and levels of insulin-like growth factor-1 (IGF-1) [41]. There appears to be a dose-dependent increase in markers of bone formation and resorption during growth hormone therapy, and this increase remains at least two years post-therapy [39].

Growth hormone therapy has been found to increase bone turnover in both normal and osteoporotic patients [39]. In healthy older men, growth hormone administration was found to increase lumbar spine bone mineral density between 0.9-1.6% [39, 42]. Skeletal sites containing a high percentage of trabecular bone, such as the lumbar spine and the greater trochanter, express increases in bone mineral density after growth hormone therapy [39].

Insulin-like Growth Factor-1. IGF-1 is a polypeptide in systemic circulation that is synthesized by the liver, muscle, adipose tissue, and bone [38, 43]. IGF-1 works closely with growth hormone to influence bone growth and remodeling [9], and research has shown that they both strongly correlate with one another [38]. Similarly to growth hormone, IGF-1 decreases with age, and the age-related changes in the growth hormone/IGF-1 axis may contribute to muscle and bone loss [38].

The relationship between IGF-1 and bone mineral density is controversial. Some of the literature states that higher IGF-1 levels are associated with greater

bone mineral densities [43], while lower levels have been found in those with osteoporosis or fractures [38, 44]. In both healthy young [45] and old [46] men, serum IGF-1 was positively correlated with bone mineral density [46]. Healthy post-menopausal women were found to have an age-related decline in IGF-1 [43] that may be associated with the on-set of menopause and with the decline in estrogens [47, 48]. However, other studies have found no relationship between IGF-1 and bone health in healthy patients, post-menopausal osteoporotic patients, or in patients with fractures [43]. However, it has been suggested that serum IGF -1 may not be a good marker of the IGF-1 in bone tissue [43].

Leptin. Leptin is a polypeptide hormone that is secreted by adipose tissue [49]. It functions to suppress appetite and increase energy expenditure [50]. Leptin is associated with inflammatory responses, its concentrations are elevated in obese subjects, and it may be partly responsible for the protective effect of fat on bone density [49, 51]. Due to the extra weight, the bones of overweight and obese subjects are subjected to increased mechanical loading during everyday activities; thus, increased loading may increase bone strength [49]. However, adiposity-associated increases in leptin production may be more strongly related to bone mineral density than the increase in weight itself [51]. Obese, leptin-deficient rats are known to weigh twice as much as their lean counterparts; however, several studies have found they also have lower bone mineral density and bone volume [51, 52]. Yet, leptin administration was found to inhibit bone loss and increase bone mineral density in leptin-deficient mice [51]. Thus, while

an increase in body weight enhances mechanical loading, it also increases leptin production, which may positively influence bone mass.

Adiponectin. Adiponectin regulates energy homeostasis and, in contrast to leptin, is reduced in obese and diabetic patients [50]. Adiponectin and its receptors have been found in osteoblasts, suggesting a relationship between the hormone and bone turnover [50]. There is a strong inverse relationship between adiponectin and bone mineral density and visceral fat [50, 53]. Zoico et al. [54] found adiponectin to have a strong negative relationship with whole body and femoral neck bone mineral density in post-menopausal women. Several authors have reported that the relationship between adiponectin and bone is stronger than the relationship between leptin and bone, such that after adjusting for fat mass, leptin was no longer significant [54, 55].

Parathyroid Hormone. Parathyroid hormone is secreted in response to low blood calcium levels [9]. Parathyroid hormone stimulates the osteoclasts to dissolve small areas of the bone, thereby releasing calcium into the blood [9]. Parathyroid hormone also inhibits calcium excretion by the kidneys, and activates vitamin D to promote absorption of calcium from the small intestine [9]. These actions work together to increase blood calcium levels. The resultant increase in calcium works as a feedback mechanism to eventually inhibit parathyroid secretion [9].

Parathyroid hormone has been shown to increase with advancing age [56]. While its effect on trabecular bone is unknown, long-term increases in

parathyroid hormone have been associated with increased loss in cortical bone, thereby reducing bone mineral density and increasing fracture risk [56]. Similarly, increases in parathyroid hormone also result in increased bone turnover and bone loss [56].

Body Composition

Body weight may explain up to 30% of the variance in bone mineral density [57], making it one of the strongest determinants of skeletal health [57-62]. Low body weight and weight loss result in decreased mechanical loading of the bones and are associated with reduced bone mineral density [59, 62, 63]. A longitudinal study conducted by Bakhireva et al. [62] found that body composition, defined as weight loss and low body mass index, was a significant predictor of bone mineral density loss in men. Greater body weight protects against bone loss due to the increased leptin secretion, mechanical stress placed on the skeleton, and potentially an increase in adipose-derived estrogen, which serves as a protectant of bone [58, 59, 62]. This increase in leptin, mechanical stress, and estrogen serves as a stimulus for osteogenesis [59].

Body weight is made up of both fat mass and lean mass, and the relationship these two components has to bone mineral density, and subsequent fracture risk, is controversial. Several studies have evaluated body composition as a means to determine the relationship between body weight and bone mineral density [57-68]. Some investigators have reported positive associations between fat mass and bone mineral density [57, 59, 62, 67], while others have found positive associations between lean mass and bone mineral density [57, 58, 60, 61, 63, 64, 67]. Lean mass is related to muscular strength, which stimulates bone remodeling through increased muscular contractions on the bone [58]; while fat mass may contribute to an overall increase in bone mineral density

through the increased hormonal effects of estrogen and leptin levels, and to the increase mechanical loading [58, 59, 62]. These are two very different findings, as the former suggests the physical activity and a healthy body weight may be protective to bone loss, and the latter suggesting that obesity is more protective [57]. Thus, while it is still unclear whether lean mass or fat mass mainly contributes to an increase in bone mineral density, it is important to note that they both contribute to an increase in body weight.

Nutrition

Adequate intakes of both macro- and micro-nutrients are required for normal bone growth and attainment of peak bone mass [24].

Anorexia Nervosa. As noted above, weight loss and a negative caloric balance are detrimental to skeletal health. Energy availability is defined as the dietary energy intake minus the exercise energy expenditure, and it represents the amount of energy available for basic body metabolism [69]. In normal, healthy, young females, the estimated energy availability should be around 30 kilocalories/kg of fat-free mass each day, and the inclusion of physical activity may raise that value closer to 45 kilocalories/kg of fat-free mass each day [69]. With that being said, an insufficient caloric intake eventually leads to suppression of menses and bone formation [70]. Many athletes, mainly female athletes, underestimate the importance of a healthy, balanced diet in maintaining general and skeletal health.

Intense exercise accompanied with an inadequate caloric intake may result in hormonal imbalances, menstrual irregularities, and bone loss. After just five days of energy restriction, the markers of bone resorption increase, while the markers of bone formation decrease [70]. Thus, the uncoupling of bone formation and bone resorption may affect the attainment of peak bone density in adolescence and may increase bone loss in adults [70].

Calcium. In bone, calcium exists as hydroxyapatite crystals, and it is the largest contributor to bone mineral content [3]. Calcium, from ingested food, is absorbed in the small intestine, and excess calcium is excreted via the kidneys [9]. Calcium homeostasis is important for the proper functioning of nerves and muscle [9]. Hormones, vitamins, and growth factors monitor and respond to any disruptions in the balance of calcium between bone and blood [9].

The hydroxyapatite crystals contribute to bone strength, and they serve as a reservoir of calcium during periods of low dietary calcium intake [3]. Majority of calcium intake comes from dairy sources, such as milk and cheese, and since bone is the primary storage reservoir for calcium, adequate calcium intake and calcium absorption are necessary for optimal bone growth [24, 71].

Vitamin D. Vitamin D is required for optimal calcium absorption [9, 24, 71]. Lower blood vitamin D levels are associated with reduced bone mineral densities and rapid bone loss from the hip and may be predictive of increased bone loss from the wrist [72]. Thus, hip fractures are more common among women with low vitamin D concentrations [73]. Since the main source of vitamin

D is from sunlight, many individuals have suboptimal vitamin D statuses. In large part, this is due to the heightened awareness of skin cancer. With the scare of melanoma, many have increased their sun-protective habits, such as the use of sunscreen, protective clothing, and staying indoors during hours of sunlight, all of which may limit the absorption of this vital nutrient, and thereby negatively impact bone mineral density.

Magnesium & Phosphorous. Both magnesium and phosphorous make up a substantial portion of bone mineral content [24]. Severe magnesium deficiency has been associated with structural changes that have a negative effect on bone volume, while excess phosphorus intake combined with a low calcium intake may negatively affect bone mineralization and bone turnover [3]. Soda beverages have been negatively associated with bone accretion in young girls, but not boys [3, 74]. Soda beverages have a low nutrient content, and may replace the consumption of nutrient-dense beverages, such as milk [74]. Adolescent girls' heel bone mineral density was significantly lower with carbonated beverage intake than young girls who did not drink carbonated beverages [74]. This significant effect was absent in adolescent boys [74]. As soda consumption increased, milk consumption reportedly dropped, and on average, girls' calcium intake was less than 900 mg of calcium a day, whereas boys' calcium intake was greater than 1000 mg per day [74]. Thus, soda consumption may lower the consumption of calcium-rich beverages, thereby affecting bone accrual at a young age.

Dairy Products. Dairy products are the primary source of dietary calcium, and those who avoid dairy products, due to intolerances, allergies, dislike, or the variety of other choices, tend to have half of the calcium consumption when compared to those whose diet includes dairy products [75]. While the calcium in dairy products is critical for bone mineralization, the other nutrients in milk, such as the other vitamins, minerals, proteins, and growth factors, may be necessary for proper bone growth and maintenance [71]. Low milk consumption during childhood has been linked to osteoporotic fractures as an adult [71]. In addition, a low consumption of dairy products leads to short stature, poor skeletal health, fractures, and a high prevalence of adiposity [71]. The current literature states that those children who avoid dairy products have low calcium intakes, poor skeletal health, and are more likely to sustain a fracture [71]; the literature also states that they have a reduced bone mineral density as adults when compared to controls [76].

There are several nutrients in foods that can alter calcium absorption [3]. A high dietary salt intake may negatively affect skeletal health, as salt is known to be the best predictor of urinary calcium excretion [3, 77]. Spinach and rhubarb contain oxalic acid, which inhibits calcium absorption [3]. Due to the calcium oxalate, the calcium in spinach is absorbed much less efficiently than the calcium in milk [3]. Similarly, phytic acid, which is the storage form of phosphorous in seeds, binds magnesium, zinc, iron, and calcium, thereby decreasing their

absorption [3]. The research is still mixed on specific foods that may increase calcium absorption [3].

Tobacco & Alcohol

The use of tobacco products and the consumption of large amounts of alcohol may have negative effects on bone health [9, 24]. It has been suggested that bone formation may be reduced in those with alcohol- or tobacco-related osteoporosis [11, 78].

Alcohol. Long-term alcohol consumption is associated with a reduced bone density and a greater risk of skeletal fractures [9]. While alcohol affects both cortical and trabecular bone, its greatest effects are on trabecular bone [9]. The effects of alcohol consumption are exerted through changes in the actions of osteoblasts, hormones, and growth factors that regulate skeletal metabolism [9].

Alcohol has been associated with reductions in bone formation and osteoblast function and increases in bone resorption and osteoclast function [9, 79]. Rat studies indicate that alcohol induces characteristics similar to that of osteoporosis, e.g. decreased trabecular bone volume, number of osteoblasts, osteoblast function, and impaired bone mineralization [9].

Alcohol consumption may negatively affect bone health by offsetting the balance of hormones that regulate calcium homeostasis [9]. Excessive alcohol intake may increase urinary calcium excretion while decreasing the secretion of parathyroid hormone; therefore, blood calcium levels are no longer optimally maintained [9]. Long-term alcohol consumption in young rats reduced bone

mineral density, bone volume, and bone strength [80]. In these rats, the longitudinal growth rate and proliferation of bone cells stopped; thereby, significantly decreasing bone mineral density [80].

Excessive alcohol intake may also interfere with the activation of vitamin D [9]. Low levels of activated vitamin D and low levels of the protein that bind to vitamin D to transport it through the blood are reduced in alcoholics [81]. This decline in vitamin D affects the absorption of calcium in the small intestine; however it is important to note that calcium levels return to normal after abstinence [82].

While excessive alcohol consumption negatively effects bone, moderate alcohol consumption (no more than 1 drink a day for women and 2 drinks a day for men) may actually aid in the reduction of osteoporosis and associated fracture risk [9]. Alcohol consumption may increase the conversion of testosterone to estradiol, which is the hormone positively associated with a reduction in post-menopausal osteoporosis [9]. Regular consumption of alcohol (5 or more days a week) reduced the risk of vertebral deformity in older women (>65 y) when compared to those that consumed alcohol less than once a week [83]. Thus, moderate alcohol consumption may not be associated with the same deleterious effects that an excessive alcohol intake has on bone health.

Tobacco. Cigarette smoking also has deleterious effects on skeletal health. Reduced bone mineral density, increased risk of hip fracture, and increased post-menopausal bone loss are more common in smokers than in non-

smokers [84]. The effect of tobacco use on bone health cumulates with age, and the negative relationship of smoking and bone health appears to be dose-dependent [84].

It is unclear whether tobacco use has a direct or indirect effect on bone. Animal studies have demonstrated that nicotine exposure impairs bone formation, which would suggest a direct effect [85]. Smoking may indirectly affect bone mineral density due to the associated reduction in calcium absorption and an increase in cortisol levels with tobacco use, as higher cortisol levels are associated with a reduction in bone mineral density [84]. The lower average body weight and the actions of tobacco on estrogen levels appear to not add any further risk to the development of osteoporosis [84]. It is important to note that smoking cessation prevents any further bone loss [84].

Diseases & Medications

Diseases known to cause osteoporosis include anorexia nervosa, celiac disease, inflammatory bowel disease, hyperthyroidism, primary hyperparathyroidism, and multiple myeloma [3, 24, 86-89]. Medications that may have a negative effect on the skeleton include glucocorticoids, chemotherapy, selective serotonin-reuptake inhibitors, anti-retroviral therapies, and anticonvulsants [3, 24, 90-92].

Anorexia Nervosa. Anorexia nervosa is a disease of "under-nutrition, low body weight, and fear of gaining weight" [3]. While this condition is more common among females, 5-15% of anorexia nervosa patients are male [3].

Anorexia nervosa is associated with a higher rate of bone resorption, bone loss, and an increased fracture risk in girls [89], and a lower bone mineral density at the lumbar spine and hip in boys [87]. The boys also had lower levels of bone resorption markers and testosterone than normal controls [87]. Thus, as noted above, achieving a daily caloric balance is vital for skeletal health.

Celiac Disease. Celiac disease is a sensitivity to gluten, a protein found in wheat, barley, and rye, that damages the lining of the small intestine and prevents absorption of nutrients from foods [3, 88, 93]. Untreated, celiac disease can lead to iron deficiency anemia, vitamin D insufficiency, low bone mass, and fractures [3, 88]. Research has shown lower vitamin D levels and lower calcium levels in children with untreated celiac disease than in controls [88]. When those with celiac disease are treated with a gluten free diet, the absorptive problems are eliminated, vitamin D levels can be corrected, and bone mineral density may improve in as little as six months [3, 88]. Due to a more active bone metabolism, the improvement in bone mineral density is stronger in children than in adults [88]. Therefore, early diagnosis and treatment is beneficial for skeletal health.

Inflammatory Bowel Disease. Inflammatory bowel disease is a group of conditions that cause inflammation of the colon and small intestine; the two main forms are Crohn's disease and ulcerative colitis [3]. Due to the inflammation, malnutrition, and variety of medications associated with inflammatory bowel disease, the risk of low bone density, and a subsequent fracture, is increased [86].

Hyperthyroidism & hypothyroidism. Hyperthyroidism is the overproduction of thyroid hormones and is known to be associated with an increased fracture risk [94]. The overactive thyroid results in accelerated bone remodeling, in which there is an increase in osteoclastic bone resorption in cortical bone [94]. The increase in osteoclast function is unproportional to the osteoblast function; thereby, increasing bone loss and fracture rate [94].

Just the opposite, hypothyroidism is an underactive thyroid gland and is associated with a higher than normal bone mineral density [94]. However, even though hypothyroid patients tend to have above normal bone mineral densities, due to inadequate remodeling, they experience an increased fracture risk [94]. The increase in fracture risk may be due to changes in bone quality, increased falls, or other unknown factors [94].

Hyperparathyroidism. Hyperparathyroidism is an overproduction of parathyroid hormones, which are known to regulate calcium and phosphate levels so the nervous and muscular systems may properly function [95]. When there is chronic overproduction of parathyroid hormone, bone remodeling increases, bone mineral density decreases, and there is an increase in fracture risk [95].

Multiple Myeloma. Multiple myeloma is a cancer of the white blood cells responsible for producing antibodies [96]. The abnormal cells accumulate in bone and bone marrow, and they interfere with the production of normal cells [96]. In patients with multiple myeloma, the normal bone remodeling cycle is

altered such that bone resorption increases out of proportion to bone formation, thereby increasing the risk of hypercalcemia, osteopenia, and subsequent fractures [96].

Glucocorticoids. Glucocorticoid-induced osteoporosis is the most frequent form of secondary osteoporosis in both genders [3]. Glucocorticoids are steroid hormones that are used to regulate glucose metabolism and to treat autoimmune, pulmonary, and gastrointestinal disorders [3]. Glucocorticoids alter osteoblast function and differentiation, thereby reducing bone formation and increasing the risk of fracture [3], which is more pronounced after age 65 [97]. Along with reduced osteoblastic function, glucocorticoids are associated with increased bone resorption, changes in muscle strength, changes in calcium absorption and excretion, and interference with growth hormone and growth factor pathways. All of these changes increase fracture risk [98]. The fracture risk associated with glucocorticoids is dose-dependent and increases dramatically after starting glucocorticoids [3]. It is important to note, fracture risk returns to baseline values after cessation of glucocorticoids; however, the reversal time is variable [3].

Chemotherapy. Chemotherapy negatively affects bone mineral density in both men and women [99, 100]. The negative effects may be due to an impairment in sex hormones, a direct effect of chemotherapy, or a direct effect of the cancer on bone [100]. Specifically, chemotherapy may directly affect the bone remodeling cycle through changes in bone resorption and bone formation

[98]. Many of the cancer therapies are associated with inhibitory effects on bone formation, which may be related to the decrease in osteoblast number that is characteristic of many chemotherapy patients [98].

Chemotherapy induces hypogonadism in both genders [98], which induces bone loss and increase fracture risk [100, 101]. This relationship between reduced gonadal function and bone is more commonly seen in estrogen-deficient post-menopausal women who have a high risk of developing osteoporosis. In pre-menopausal women, chemotherapy induces premature menopause; therefore, the increased bone loss seen in pre-menopausal cancer patients may be related chemotherapy-induced ovarian failure [102]. Due to increased follicle-stimulating hormone and luteinizing hormone levels characteristic of impaired Leydig cell function, male chemotherapy patients also have reduced bone mineral density of both cortical and trabecular bone [100]. Thus, in men and pre- and post-menopausal women chemotherapy is associated with deleterious effects on bone [100].

Selective Serotonin-Reuptake Inhibitors. Selective serotonin-reuptake inhibitors (SSRIs) are used to treat depression, other psychiatric disorders, and chronic pain [91]. They block the serotonin transporter, which is located in the central nervous system, periphery, and bone, to increase the extra-cellular concentration of serotonin [91]. SSRIs have a negative effect on bone mineral density and increase fracture risk in both men and women [91]. The bone mineral density differences between those who use SSRIs and controls ranges

from 2.4 to 6.2% at all anatomical sites [91]. The literature suggests a 1.5-fold increase in fracture risk for every unit increase in the daily dose of a SSRI [103], and this increase is maintained for at least 5 years [104]. Similar to other medications that affect bone, SSRIs reduce bone formation and increase bone resorption, leading to frail, fragile, fracture-prone bones [91].

Anti-Retroviral Therapy. The literature suggests that anti-retroviral drug therapies may negatively alter bone metabolism [92]. Anti-retroviral therapies are used to control HIV, and they have been successful in extending the life span of patients with AIDS and in reducing the rate of AIDS-related mortality [92]. A high incidence of osteoporosis and osteopenia has been reported in those on anti-retroviral therapies [92]. A study conducted on AIDS patients receiving anti-retroviral therapies found osteopenia in 50% and osteoporosis in 21% of anti-retroviral users [105]. These values were significantly higher than the non-treated patients and controls [105]. The large reduction in bone mineral density is thought to be associated with the inhibition of osteogenesis and reduction in osteoblast function and recruitment commonly seen with anti-retroviral therapeutic interventions [92].

VII. Sex Steroids

As mentioned above, there are many hormones that regulate normal bone metabolism, and sex steroids are particularly important for skeletal growth and maintenance [3, 31, 106]. Changes in their levels can lead to suboptimal peak bone mass during adolescence or can directly lead to bone loss as an adult

[107]. It is well-known that aging-associated bone loss in both men and women is related to an increase in sex hormone binding globulin (SHBG) and the associated decreases in bioavailable levels of testosterone and estrogen [108].

Sex Hormone Binding Globulin

Both testosterone and estrogen can be found bound to SHBG, bound to albumin, or free [108]. The proportions bound to SHBG are no longer available to access target tissues; however, the free proportions and the albumin-bound proportions of testosterone and estrogen are termed "bioavailable" and can act on target tissues, like bone [108, 109].

Throughout the lifespan, SHBG increases and binds to a greater proportion of the bioavailable sex hormones [110]. Typically, total testosterone and total estrogen remain unchanged in men throughout the lifespan; while estrogen levels decline after menopause in women, their total testosterone levels remain relatively unchanged [110]. However, as one ages SHBG binds to greater proportions of the bioavailable sex hormones, thereby decreasing the bioavailable testosterone and estrogen and reducing their effect on target tissues [110]. So while testosterone and estrogen levels never truly change, the proportions that actively stimulate bone and muscle are decreasing throughout life.

The binding of SHBG to estrogen and testosterone reflects the increased risk of fracture associated with lower concentrations of these hormones in the "free" or bioavailable state [73, 111]. However, Stone et al. [111] suggests that

SHBG may influence bone in ways other than the binding of sex steroid hormones. The concentration of SHBG increases with advancing age, and has been found to be an independent predictor of femoral neck and lumbar spine bone mineral density, even after controlling for levels of testosterone and estrogen [56]. In women, SHBG has been associated with changes in bone mass, bone loss, and hip and vertebral fractures, and has been found to be higher in those with osteoporotic fractures than controls [56]. However, while the relationship between testosterone and estrogen and bone mass appears to be bone-preserving, SHBG appears to have a bone-wasting effect [112]. Thus, it is suggested that increased levels of SHBG cause bone loss and increase the risk of an osteoporotic fracture [112].

While it may not be as good a predictor of bone mineral density as estradiol, SHBG is a significant predictor of bone mass in both genders [111]. In women, SHBG has been associated with changes bone mass, bone loss, and hip and vertebral fractures [73, 111, 112]. The combination of undetectable estrogen levels combined with a high SHBG concentration (1 ug/dl or greater) is associated with a very high fracture risk [73]. In older men, as SHBG increases, there appears to be an associated decrease in muscular strength, bone density, body composition, testosterone, and estrogen [109]. Thus, SHBG mainly affects bone density by decreasing the bioavailable proportion of testosterone and estrogen; however, SHBG may exert independent effects on bone as well.

Testosterone

Due to the awareness of menopause-associated bone loss, much of the research on sex steroid hormones and bone has focused on estrogens; however, testosterone also has an important role in lean body mass and skeletal development and maintenance in both genders. The current literature has associated androgens with longitudinal bone growth during development, regulation of trabecular and cortical bone mass, peak bone mass acquisition, and inhibition of bone loss [3]. Due to androgen receptors on bone, testosterone may have a direct effect on bone mass. However, testosterone-associated changes in lean body mass, muscle mass, and strength may have indirect effects on bone [108].

Bone Mineral Density. In young women and men, normal testosterone levels are important in attaining peak bone mass and maintaining bone mass after puberty [56]. Since 50% of men with hip fractures and 20% of men with vertebral fractures have been reported to be hypogonadal, low testosterone levels have been associated with osteoporotic fractures [56]. However, the current research has failed to come to a conclusive relationship between testosterone levels and bone mineral density in either gender.

In women, the research on the impact testosterone has on bone health is conflicting at best. In peri- and post-menopausal women, a weak correlation between testosterone and vertebral bone mineral density was been reported [113]. In another study, total testosterone was not correlated with bone mineral density, but free testosterone was related to the change in femoral neck bone

mineral density [114]. Greendale et al. [106] did not find any relationship between total testosterone and bone mineral density, but did find an association between free testosterone and bone mineral density at all measured sites.

While a greater proportion of the research on testosterone and bone density has been conducted in men, there is still a lack of conclusive evidence. In elderly men, Drinka et al. [115] did not find a relationship between bioavailable testosterone and bone mineral density at any site. According to Van Den Beld et al. [109], free and albumin-bound testosterone levels are related to proximal femur bone mineral density, while total testosterone levels were not. While Murphy et al. [116] found a significant relationship between total testosterone and bone density at the spine and hip in men ranging in age from 21 to 79 years.

Conversely, another study of older men found a positive relationship between bioavailable testosterone and bone density at the ultradistal radius, spine, and hip [106]. These subjects had normal testosterone levels, and the intramuscular testosterone administration increased bone mineral density by 5%, and simultaneously, increased estrogen levels by 45% [106]. The increase in bone mineral density was significantly related to the increase in estrogen levels, but not with the increase in testosterone [106]. Thus, it was hypothesized that the increase in bone mineral density was due to the amortization, or the conversion, of testosterone to estrogen [106]. Therefore, it appears that total testosterone levels are not related to bone health in men, but free and

bioavailable testosterone levels may influence bone at specific sites, and this influence may be directly related to the conversion of androgens to estrogens.

Amortization of Androgens to Estrogens. Some of the current research points to a stronger relationship between estrogen and bone density than testosterone and bone density [56]. However, the importance of testosterone cannot be overlooked. It is through the amortization of testosterone that estrogens are created. Aromatase is an enzyme system that catalyzes the conversion of androgen precursors into estrogens [107]. In human bone, aromatase is expressed in osteoblasts, chondrocytes, adipocytes near bone trabeculae, and in osteocytes, but not in osteoclasts [107]. In men, 85% of the circulating estrogens come from the amortization of androgens to estrogen, while the remaining 15% of the circulating estrogens comes directly from the testes [107].

In the current literature, the importance of estrogens in the regulation of bone growth and maintenance is apparent; however, the amortization of androgens into estrogen for male bone health is recognized as important [107]. A small number of men have a mutation in the aromatase gene [3, 31, 56, 107]. These men all grew to be very tall due to continued longitudinal growth, had unfused epiphyses, delayed bone age, lack of a pubertal growth spurt, elevated markers of bone turnover, and had severe osteopenia [3, 107]. Since these men had normal testosterone levels, testosterone therapy did not confer any benefit. However, estrogen therapy was found to significantly increase bone mass,

increase bone size, suppress bone resorption, cease longitudinal growth, and quickly close the epiphyseal growth plates; thereby suggesting a role for estrogen in skeletal maturation and mineralization in men [3, 31, 56, 107]. Thus, these case studies suggest an important role for testosterone, estrogen, and the aromatase gene in skeletal health.

Studies of aromatase knockout mice or rats treated with an aromatase inhibitor have helped to outline the importance of aromatase in the male and female skeleton. In the male rats, the inhibition of aromatase leads to the inhibition of estrogen production, increased bone resorption, and bone loss [107]. When compared to wild type mice, male aromatase knockout mice had a reduced femur length growth and did not experience the growth spurt during puberty; while female aromatase knockout mice had no changes in femur length [107, 117]. However, both male and female knockout models had osteopenia [117].

Under estrogen therapy, the aromatase knockout mice were able to achieve the same femoral neck bone mineral density as their wild type counterparts, probably due to the increase in bone remodeling [118]. The untreated aromatase knockout mice had reduced bone mineral density, particularly in areas with a high percentage of trabecular bone [118].

Lean body mass. It is well-known that lean body mass, muscle mass, and strength decline with advancing age, and this decline is in direct proportion to the bioavailable testosterone [108]. The decrease in lean muscle mass, strength,

and power associated with declining testosterone concentrations is caused by the atrophy of muscle fibers [108]. Testosterone binds to the androgen receptor in the muscle and stimulates protein synthesis resulting in muscle fiber hypertrophy; thus, reduced bioavailable testosterone levels are detrimental to lean body mass and strength, which could lead to reduced contraction forces on to the skeleton [110]. These reduced tensile forces may impact bone mineral density, as many studies have reported a positive relationship between bone mineral density and serum testosterone levels [109]. On that same note, current research suggests that testosterone administration leads to a dose-dependent increase in bone density, lean body mass, muscle size, and strength [108, 109].

The relationship between estrogen and bone mass in women is widely recognized, however, the relationship between testosterone and bone mass and lean body mass in post-menopausal women is under-researched [108]. However, a study in middle aged and elderly women found a significant relationship between bioavailable testosterone and muscle mass and muscle strength [119]. It has also been reported that post-menopausal women using estrogens had reduced levels of bioavailable testosterone and this resulted in a loss in lean body mass [120]. While very few studies have researched the effects of testosterone on bone in women, van Geel et al. [108] found that the age-associated loss of lean body mass and bone mineral density in post-menopausal women is dependent on the concentrations of both bioavailable estrogen and testosterone. Thus, the decrease in lean body mass and bone

mineral density with advancing age may be related to reduced testosterone and estrogen concentrations.

Up until the early 1990's, it was thought that estrogens played a strong skeletal role in women, and testosterone was important in male bone metabolism [3]. However, this next section focuses on the recognition of estrogen as an influential factor for bone mineral density in both genders.

Estrogen

It is well-known that estrogens play an important role in bone health, as one of the more researched risk factors for osteoporosis is the declining concentration of estrogen in post-menopausal women [3, 24]. It has been suggested that estrogen deficiency may be responsible not only for the rapid reduction in post-menopausal bone loss, but also for the slow age-related decline in bone mass seen in both men and women [31]. The loss of bone mass after menopause and the maintenance of bone mass with use of estrogen after menopause suggest a strong relationship between estrogen and bone health in women, and the strength of this relationship is becoming more prominent in men. In both men and women, bioavailable estrogen is the sex steroid that has the strongest association with bone mineral density [106]. In post-menopausal women, estrogen therapy has been found to prevent secondary hyperparathyroidism and the increased bone turnover associated with aging [121]. Thus, estrogen has been associated with intestinal calcium absorption,

renal calcium handling, bone turnover, and may prevent the age-related increase in parathyroid hormone [31].

In men, total estrogen levels remain somewhat stable throughout the lifespan; however, the bioavailable form of estrogen decreases with advancing age [31, 56]. There are several possibilities for the age-related decrease in estrogen, including a decline in the aromatization substrate that converts testosterone to estrogen, the age-related increase in SHBG, or the ability to aromatize estrogen from testosterone may change with age [122]. To test the theory that the ability to aromatize testosterone to estrogen changes with age, Kholsa et al. [122] examined the ratio of estrogen to testosterone in young, middle-aged, and elderly men. They found that the ratio increased with advancing age, suggesting that the ability to aromatize testosterone to estrogen increases with age [122]. Thus, the increase in SHBG may hold an important role in the decline of bioavailable estrogen.

In healthy elderly men, estrogen has an important role in the maintenance of bone density. Serum total estrogen was found to have a strong positive association with bone mineral densities at all sites; while free estrogen was found have a slightly stronger relationship with bone mineral density when compared to total estrogen levels [106, 109]. Research in aromatase deficient men has suggested that estrogens are critically important in controlling the rate of bone remodeling in the male skeleton [107].

Conversely, in women, estrogen levels remain relatively stable until menopause, when the ovaries dramatically reduce estrogen levels to those less than half of aging men [31]. This dramatic decrease in estrogen levels during menopause triggers a rapid period of bone loss that is not seen in men. Much of the research shows that there is a threshold estrogen concentration that must be met for normal skeletal remodeling [107]. Even though aging men tend to have estrogen levels that are at least two-fold greater than those of post-menopausal women [106], this threshold value is much higher than the average estrogen levels in post-menopausal women and in aging men. However, premenopausal women and young men are usually well-above this range [107]. Cummings et al. [73] found that post-menopausal women with estrogen concentrations less than 5 pg/ml have an increased risk of hip and vertebral fracture. Thus, the menopause-associated drop in estrogen levels most likely accounts for the two-fold greater bone loss over the lifespan in women than men [31]. Therefore, total and bioavailable estrogen levels decrease in women due to a decrease in ovarian estrogen production, and they decrease in men due to an age-related increase in SHBG.

Thus, estrogens are no doubt important for female and male skeletal metabolism, and estrogen receptors have been found in osteoblasts and osteoclasts from both genders [56]. As mentioned, bioavailable estrogen levels are positively correlated with bone mineral density, and they are a consistent independent predictor of bone mineral density in elderly men [31]. The current

literature has suggested that estrogen is the best predictor of the attainment of peak bone mass in young men and the decrease of bone mass in elderly men [31, 106, 122, 123]. Khosla et al. [31] found similar relationships between bioavailable estrogen and bone mineral density in both genders, such that bone resorption is enhanced, thereby suggesting a similar role for estrogen in determining bone mineral density in both men and women. However, the same relationship was not seen in pre-menopausal women; thus, suggesting that estrogen levels above a certain threshold are not associated with bone mineral density [31].

The importance of estrogens in preserving bone in post-menopausal women has been confirmed by studies in which estrogen therapy improves fracture risk and prevented bone loss [111, 124, 125]. With that being said, high levels of estrogens and androgens have been found to prevent or reverse the increased bone turnover and prevent bone loss in this age group [31, 111]. When women had estrogen levels 10pg/ml or greater, no significant loss in bone mass was observed; however, women with undetectable levels of estrogen (less than 5pg/ml) were found to lose hip bone mineral density at a rate of 1% per year [111].

In both men and women, bioavailable estrogen was found to be more strongly associated with bone mineral density than bioavailable testosterone [106, 122]. However, most of the estrogen produced in normal men is formed by the amortization of androgens; thus, serum testosterone is important for the

maintenance of estrogen concentrations, and testosterone therapy may actually increase estrogen levels [31, 126]. Anderson et al. [126] found that in men with osteoporosis, testosterone therapy increased lumbar spine bone mineral density. The increase in bone mineral density was correlated with the testosterone-induced increase in estrogen and not the increases in serum testosterone levels themselves [126]. Yet as noted above, the effect of testosterone on bone may not only be attributed to its amortization to estrogens, but through increases in lean body mass, strength, and bone remodeling [109]. Thus, even though estrogen is more strongly associated with bone mass in both men and women, testosterone and estrogen therapies are both beneficial to bone health [31, 126].

The complete absence of estrogen is associated with increased rates of bone resorption and osteocyte apoptosis [73]. Osteocytes have an important role in the skeletal response to bone damage and physical activity-induced strains; thus, the lack of a response is detrimental to bone health [73]. Dunstan et al. [127] reported that osteocyte death is characteristic of elderly women with hip fractures. It is believed that estrogen protects against osteocyte apoptosis [123], and estrogen deprivation may increase osteocyte apoptosis [128]. Therefore, after menopause, estrogen levels plummet and osteocyte apoptosis is on the rise. The increase in osteocyte death triggers the activation of osteoclasts, and overall bone resorption is increased.

Estrogen deficiency is the main cause of rapid post-menopausal bone loss, not to mention that it contributes to bone loss associated with aging [24].

Estrogen inhibits bone resorption, thereby reducing bone loss at all skeletal sites [24]. In post-menopausal women or in women with declining ovarian function, cancellous bone mass is in jeopardy [3]. Women with undetectable serum estrogen concentrations (less than 5pg/ml) have an increased risk of fracture; it has been suggested that the risk of fracture can be reduced with estrogen therapy, especially if estrogen therapy begins shortly after menopause [73]. Estrogen therapy has been shown to reduce hip fractures by 50% and vertebral fractures by 90% [24].

Estrogen deficiency may also lead to an increase in several cytokines (interleukin-6, interleukin-7, and tumor necrosis factor-alpha) that stimulate osteoclastic activity [10]. Due to the increase in the osteoclast lifespan and bone resorption, the composition and maintenance of trabecular bone suffers, thereby accelerating bone remodeling and bone loss [10]. This type of bone loss can reach -2% within a year, leading to a reduction of 20-30% of initial bone mass throughout the menopausal period [10]. Thus, while estrogen used to be mainly thought of as a vital hormone for female bone health, it is now being recognized as an important regulator for skeletal metabolism in both sexes, and is more strongly associated with bone mass than other sex steroid hormones.

VIII. Physical Activity

A lack of physical activity is a modifiable risk factor for osteoporosis; however, to obtain the skeletal benefits of physical activity, at least a small proportion must be weight-bearing in nature [63, 129, 130]. While several

studies have shown that including a modest amount of weight-bearing activity in one's workout regime is necessary to gain the full benefits in terms of bone health [129, 130], the specific type of physical activity that best promotes bone strength is unclear. It is currently believed that there are three guidelines for osteogenic physical activity: 1) the activity is dynamic, as opposed to static; 2) a loading that is of short duration, and 3) the activity must be "abnormal," due to the skeletal accommodation of "normal" strains [131]. It is also believed that the insertion of rest periods in between loading bouts is important for the bone to optimally adapt to the loading [30].

Dynamic Strain

For well-over a century, it has been known that the stresses placed upon the bone determine its strength and architecture [131]. In the middle of the twentieth century, Frost et al. [132] determined that while stresses placed upon the bone are important for bone adaptations, the stresses much achieve a minimum effective strain before the adaption could occur. Thus, the bone deformation must reach a certain point before bone is added; likewise, if the stresses fall below a certain point, bone is lost [3]. Less than a decade later, it was found that dynamic, as opposed to static, strains increased bone strength [131]. More recently, it was determined that the frequency of loads also influenced bone adaptions [30, 133]. Thus, the greatest bone adaptions happen with dynamic strains, at a magnitude or frequency that can be detected by the

bone cells [30]; this increases the strain rate, and therefore, directly influences bone strength.

Short Duration

Longer bone-loading exercise bouts do not proportionally increase the bone formation response [131]. The detection of the mechanical signal requires that the bone cells be in a sensitive, receptive state [30]. Bone cells quickly desensitize to mechanical stimuli; such that further mechanical signals, which would normally be osteogenic, are irrelevant to the cell [30]. Animal limb bones that underwent 10-50 load cycles a day exhibited a large gain in bone mineral density [30]. When the load was increased to greater than 50 load cycles a day, the bone mineral density changes were insignificant. Thus, bone cells may respond to the first 50 load cycles, but if the mechanical loading continues, the bone cells become unresponsive, diminishing the cellular response [30].

Similarly, Robling et al. [134] found that rat ulnas increased osteogenesis almost twice as much if load cycles were separated into 4 bouts of 90 cycles, each separated by 3 hours, compared with 1 bout of 360 cycles. After 16 weeks, the ulnas exhibited a 5.4% and 8.6% increase in bone mineral density [134]. The two protocols (4 bouts x 90 cycles, 1 bout x 360 cycles) received the same total number of cycles per day (360), delivered at the same force magnitude and frequency [134]. Thus, the bone mass differences cannot be explained by differences in mechanical input. Rather, the inclusion of a 3 hour rest period in between cycles allowed for the bone cells to recover some of the lost sensitivity;

therefore, they were more sensitive to the mechanical loading throughout all subsequent bouts [134]. When exposed to 1 bout of 360 cycles, the bone cells had desensitized after 50 or so cycles, and did not respond to the later cycles [134]. Thus, short load cycles separated by rest periods may increase the sensitivity of the bone cells and increase the osteogenic response.

Bone Accommodation

"Abnormal" stresses applied to the bone drive adaptive changes [131]. The stresses that induce osteogenesis are not the numerous cycles of normal stresses that the bone experiences on a day-to-day basis; rather, the strains that induce bone formation are the "abnormal" stresses that are experienced during activities to which the bone is not accustom [131, 135]. Thus, the bone cells become accustomed to the strains associated with repetitive activities, such as walking or running. However, activities, such as multi-directional jumping, that produce strains to which the bone has not come to expect can produce skeletal changes that enhance the bone formation response.

Muscular Contractile Forces & Gravitational Loading

Similar to muscle, bone is influenced by mechanical forces [136]. Removal of these forces causes bone loss, reduced bone formation, and increased bone resorption, resulting in an increase in fragility fractures [136]. However, increased mechanical loading can increase bone formation and decrease bone resorption [136].

It was previously thought that during exercise, the muscle contracts and pulls on the bone at the attachment site [137]. This momentary pull on the bone stretches the osteocytes and causes a cascade of events to result in an adaptation of the bone [137]. However, for this to occur, the degree to which the osteocytes would need to be stretched would be too great for the bone to bear [136]. Thus, the current theory points to extracellular fluid flow in the bone tissue that causes the bone to adapt to the mechanical loads [137]. The extracellular fluid makes up about 23% of the bone volume and it flows outward along cortical bone [136]. When a compressive force (muscle contraction) or an impact force acts on the bone on one side, the bone pushes water out the other side, much like a sponge [138]. The velocity of the extracellular fluid is dependent on the applied mechanical force [131, 138, 139]. In culture, osteoblasts and osteoclasts have both responded to this fluid sheer stress and it has shown to affect bone metabolism [140]. With that being said, dynamic exercise, as opposed to static exercise, has been shown to elicit a large fluid shear stress and therefore a greater osteogenic response [138].

The two different forces that elicit mechanical loads and create fluid sheer stress are muscle contractile forces and gravitational loading [137, 141, 142]. These forces influence bone shape, strength, and size through exercise. Most activities, if not all, that involve gravitational loading also involve muscle forces. However, there are some activities that only involve muscle forces, those being the non-weight-bearing sports, such as swimming and cycling. It is unclear

whether stronger muscle contractions or greater impact loads induce the greatest fluid sheer forces, but cross-sectional studies that have analyzed the bone mineral densities difference among various weight-bearing and non-weight-bearing sports have begun to separate the osteogenic effects of muscle contraction forces and impact forces on bone strength.

Weight-Bearing & Non-Weight-Bearing Exercise. Many cross-sectional studies have examined bone mineral densities in various sports. In regards to long-distance runners and cyclists, the gravitational load associated with running has helped to conclude that weight-bearing exercise is more osteogenic than non-weight-bearing exercise [63, 143]. With that being said, several studies have found high prevalence rates of osteoporosis and osteopenia in adult male road cyclists [63, 130, 143-145], but not in distance runners [63, 143]. However, distance running has a negative-dose-dependent relationship with bone mass; the longer the distances, the lower the bone mass [146]. A study comparing runners, cyclists, weight lifters, and controls, showed that the cyclists and controls had similar bone mineral densities at all sites; whereas the weight lifters and runners had significantly higher bone mineral densities [147]. Another study that compared cyclists and age-matched sedentary controls found that the cyclists had lower bone mineral densities than the controls, but when weight was accounted for, the differences were obsolete [148].

After comparing the effects of cycling and running on bone mineral density, it has been concluded that a lack of weight-bearing exercise is harmful to

bone health [143]. The low bone mineral densities found in many cyclists may be attributed to the horizontal distribution of body weight or the failure of cycling to produce skeletal strains [143]. Therefore, cycling has been suggested to be negatively associated with skeletal health, whereas running has been positively associated with bone mineral densities at weight-bearing sites [143]. Since runners and cyclists both undergo significant muscle loading, it may be the impact forces, measured via ground reaction forces, which account for the primary skeletal differences. For athletes that participate in both running and cycling, it is important to note that running may counteract the negative effects of cycling [143].

Non-endurance weight-bearing sports have been associated with greater bone mineral densities than endurance weight-bearing sports. Rector et al. [149] found weight-trainers to have an increased bone mineral density when compared to runners, and runners to have an increased bone mineral density when compared to cyclists. However, the weight-trained athletes had greater bone mineral density than the reference population, and cyclists were found to have a reduced bone mineral density when compared to the reference population [149]. This seems to suggest that weight-bearing exercise is more osteogenic than both weight-bearing endurance exercise and non-weight-bearing exercise.

Sports with a high force loading, such as soccer, volleyball, and gymnastics are associated with stronger and more dense bones [3]. The athletes in the highest-impact sports (i.e. basketball and volleyball) have been

found to have the greatest levels of bone formation markers and bone mineral densities when compared to moderate-impact sports (i.e. soccer and track), no-impact sports (i.e. swimming), and sedentary controls [129]. The athletes involved in the non-impact sports were found to have a reduced bone mineral density at the hip than the high- and moderate-impact sports but not different from the controls [129]. Thus, the stress associated with high-impact, weight-bearing sports may induce bone formation and enhance osteogenesis at weight-bearing sites.

Muscular contraction forces and gravitational loading are intertwined and the effects of each are difficult to separate. Studies that have tried to separate the effects of each will be discussed next.

Muscle Contraction Forces

It is well-accepted that bone adapts to the mechanical demands it is subject to, and muscle contractions contribute a portion of those demands [3, 137]. The literature has demonstrated that the gain and decline of bone mass is consistent with the gain and decline of muscle strength throughout life (**Figure 23**) [137]. With that being said, in states of muscular disuse, such as disease, inactivity, paralysis, etc., the muscle forces are severely reduced and there is a lack of skeletal strain, thereby causing a site-specific reduction in bone mass and bone strength [3]. The effect of muscle contraction forces can be separated from gravitational loading by observing the bone adaptions in non-weight-bearing sports, observing the upper limb and/or unilateral training studies, and paralysis studies.

Upper Limb. The upper limb undergoes significant muscle contractions, but since it does not transmit body weight to the ground, it is not subjected to the effects of gravitational loading [137]. Heinonen et al. [150] conducted a 12-month unilateral upper limb resistance training and 8-month detrain study. Even though strength improved in the trained arm, bone mineral density did not improve in either the trained arm or the untrained arm [150]. During detraining, muscle strength was lost in both limbs, but there was no change in bone mineral density [150].

Conversely, studies on tennis players, in which the racket arm is compared to the non-racket arm, demonstrate that the racket arm has increased

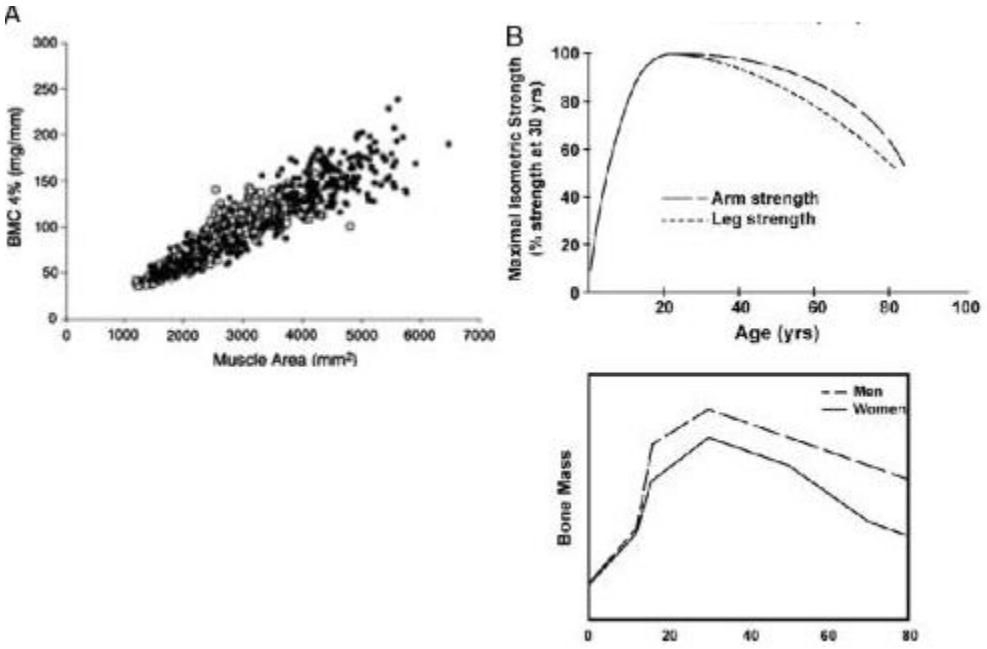


FIGURE 23. Adapted from Robling et al. [137]. A) There is a close association between forearm muscle area and bone mineral content in the distal radius. B) Isometric muscle strength and bone mass show a very similar profile over a large age range.

muscle and bone mass than the non-racket arm [151]. On the same note, gymnasts have been found to have higher arm bone mineral density than controls [152]. The side-to-side variations in arm bone mineral density between female tennis player and controls was found be between +5.8% to +22.5% [153]; and male tennis players were found to have a 25.4% greater bone mineral density in their playing arm as opposed to their non-playing arm [154]. While tennis players clearly do not experience gravitational loading in their arms, the impact forces or the increased muscle mass associated with the playing arm may contributed to increased bone mineral density and skeletal health.

Unilateral Lower Body Resistance Training. While the lower limbs transmit body weight to the ground and are subjected to ground reaction forces, unilateral lower limb workouts may still successfully separate out the effects of muscular and impact forces on bone strength. Subjects that underwent a unilateral one-year resistance training study, in which they performed the leg press (left leg) four times a week had a significant increase in left leg muscle strength [155]. The trained leg was trending toward a significant increase in the femur, patellar, and proximal tibia bone mineral density [155]. The untrained leg (right leg) experienced a cross-training effect such that it increased in muscle strength, and saw a very small, non-significant increase in bone mineral density [155]. During an eight-month detraining period, the increased muscular strength remained in both the trained and untrained leg, while any increases in bone mineral density were back to baseline within 3 months [155].

Intrauterine-Onset Neuromuscular Paralysis. Infants born with intrauterine-onset neuromuscular paralysis exhibit normal bone length, but impaired cortical thickness and bone mass [137]. Due to low bone mass, these infants are typically born with many fractures that occurred during gestation [137]. Since fetuses are in a weightless environment, there is a lack of gravitational loading influencing bone mass [137]. Thus, it is the lack of muscle contractions in the paralyzed infants that causes the bones to become thin and fragile [137].

Thus, the studies on muscular contractions and bone density demonstrate that while muscular contractions are important for bone strength, it may be the combination of both muscular contractions and impact forces that induce positive skeletal adaptations.

Gravitational Loading

Gravitational loads are reactive loads resulting from contact between a weighted body (i.e. human) and a substrate (i.e. ground) [141]. Gravitational loads are measured via ground reaction forces and are determined by the body mass and the acceleration/deceleration of the activity [141]. During high-impact activities, such as gymnastics, the ground reaction forces can be between 10 and 20 times the body weight [141]. Weight-bearing activities increase both mechanical loading and gravitational loading. It is nearly impossible to separate the two, and it remains to be determined whether mechanical or gravitational loading is the dominant osteogenic stimulus [141].

However intertwined gravitational and mechanical loading may be, current research has suggested that gravitational loading has a powerful influence on skeletal health [141]. For example, during space flight, the astronauts experience up to 3% bone loss in one month [156]. Since the astronauts are still actively using their muscles during everyday activities, the elimination of gravity and impact forces must dramatically affect bone health [156]. Similarly, there is an accelerated rate of bone loss during bed rest [142]. Therefore, the findings from space flight and bed rest studies indicate that high-impact forces may be

necessary to preserve bone mineral density at weight-bearing sites [129]. Thus, gravitational forces play a role in skeletal health.

As noted above, the impact forces associated with gravitational loading are not present in non-weight-bearing activities, and it may be these impact forces that stimulate osteogenesis. In activities such as distance running and high-impact sports, it has been proposed that the ground reaction forces stimulate bone turnover, and therefore increase the bone resorption and formation markers in blood [63]. The mechanical stress associated with gravitational loading is not seen in cyclists or swimmers, as neither sport is weight-bearing [66]. However, both cyclists and swimmers experiences large muscle forces. With that being said, the low levels of impact seen in non-weight-bearing activities negatively influences bone formation and bone mineral density [66, 129]. Thus, while muscle is important in skeletal strength and health, bone also relies on impact loads to generate sufficient loads that meet the frequency and magnitude required for bone adaptations.

REFERENCES

1. Brown, S.A. and C.J. Rosen, *Osteoporosis.* Med Clin North Am, 2003. **87**(5): p. 1039-63.

2. Augat, P. and S. Schorlemmer, *The role of cortical bone and its microstructure in bone strength.* Age Ageing, 2006. **35 Suppl 2**: p. ii27-ii31.

3. Eric S. Orwoll, J.P.B., Dirk Vanderschueren, ed. *Osteoporosis in Men: The Effects of Gender on Skeletal Health* 2nd ed. 2010, Elsevier Inc. .

4. Favus, M.J., ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Vol. 6. 2006, American Society for Bone and Mineral Research: Chicago.

5. Copstead, L.C., ed. *Perspectives on Pathophysiology*. 1995, WB Saunders: Philadelphia.

6. Carter, D.R. and D.M. Spengler, *Mechanical properties and composition of cortical bone.* Clin Orthop Relat Res, 1978(135): p. 192-217.

7. Gomez, S., *Crisostomo Martinez, 1638-1694: the discoverer of trabecular bone.* Endocrine, 2002. **17**(1): p. 3-4.

8. Goldstein, S.A., *The mechanical properties of trabecular bone: dependence on anatomic location and function.* J Biomech, 1987. **20**(11-12): p. 1055-61.

9. Sampson, H.W., *Alcohol's harmful effects on bone.* Alcohol Health Res World, 1998. **22**(3): p. 190-4.

10. Chappard, D., et al., *Trabecular bone microarchitecture: a review.* Morphologie, 2008. **92**(299): p. 162-70.

11. Legrand, E., et al., *Trabecular bone microarchitecture, bone mineral density, and vertebral fractures in male osteoporosis.* J Bone Miner Res, 2000. **15**(1): p. 13-9.

12. Bethel, M., E.F. Srour, and M.A. Kacena, *Hematopoietic cell regulation of osteoblast proliferation and differentiation.* Curr Osteoporos Rep, 2011. **9**(2): p. 96-102.

13. Mackie, E.J., et al., *Endochondral ossification: how cartilage is converted into bone in the developing skeleton.* Int J Biochem Cell Biol, 2008. **40**(1): p. 46-62.

14. Adams, C.S. and I.M. Shapiro, *The fate of the terminally differentiated chondrocyte: evidence for microenvironmental regulation of chondrocyte apoptosis.* Crit Rev Oral Biol Med, 2002. **13**(6): p. 465-73.

15. Shapiro, I.M., et al., *Fate of the hypertrophic chondrocyte: microenvironmental perspectives on apoptosis and survival in the epiphyseal growth plate.* Birth Defects Res C Embryo Today, 2005. **75**(4): p. 330-9.

16. Henriksen, K., et al., *Local communication on and within bone controls bone remodeling.* Bone, 2009. **44**(6): p. 1026-33.

17. Matsuo, K. and N. Irie, *Osteoclast-osteoblast communication.* Arch Biochem Biophys, 2008. **473**(2): p. 201-9.

18. Mulari, M.T., et al., *Osteoblast-like cells complete osteoclastic bone resorption and form new mineralized bone matrix in vitro.* Calcif Tissue Int, 2004. **75**(3): p. 253-61.

19. Everts, V., et al., *The bone lining cell: its role in cleaning Howship's lacunae and initiating bone formation.* J Bone Miner Res, 2002. **17**(1): p. 77-90.

20. Hayden, J.M., S. Mohan, and D.J. Baylink, *The insulin-like growth factor system and the coupling of formation to resorption.* Bone, 1995. **17**(2 Suppl): p. 93S-98S.

21. Mundy, G.R. and L.F. Bonewald, *Role of TGF beta in bone remodeling.* Ann N Y Acad Sci, 1990. **593**: p. 91-7.

22. Bikle, D.D., et al., *Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone.* J Bone Miner Res, 2002. **17**(9): p. 1570-8.

23. Walker, E.C., et al., *Cardiotrophin-1 is an osteoclast-derived stimulus of bone formation required for normal bone remodeling.* J Bone Miner Res, 2008. **23**(12): p. 2025-32.

24. *Consensus development conference: prophylaxis and treatment of osteoporosis.* Osteoporos Int, 1991. **1**(2): p. 114-7.

25. Organization, W.H., *WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level* 2004, World Health Organization Brussels

26. Kanis, J.A., et al., *The diagnosis of osteoporosis.* J Bone Miner Res, 1994. **9**(8): p. 1137-41.

27. Haentjens, P., et al., *Meta-analysis: excess mortality after hip fracture among older women and men.* Ann Intern Med, 2010. **152**(6): p. 380-90.

28. Szulc, P., *Bone density, geometry, and fracture in elderly men.* Curr Osteoporos Rep, 2006. **4**(2): p. 57-63.

29. Melton, L.J., 3rd, et al., *Long-term fracture prediction by bone mineral assessed at different skeletal sites.* J Bone Miner Res, 1993. **8**(10): p. 1227-33.

30. Robling, A.G., et al., *Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts.* J Bone Miner Res, 2002. **17**(8): p. 1545-54.

31. Khosla, S., et al., *Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen.* J Clin Endocrinol Metab, 1998. **83**(7): p. 2266-74.

32. Scott Powers, E.H., ed. *Exercise Physiology: Theory and Application to Fitness and Performance*. 7th ed. 2008, McGraw-Hill.

33. Davies, K.M., et al., *Prevalence and severity of vertebral fracture: the Saunders County Bone Quality Study.* Osteoporos Int, 1996. **6**(2): p. 160-5.

34. O'Neill, T.W., et al., *The prevalence of vertebral deformity in european men and women: the European Vertebral Osteoporosis Study.* J Bone Miner Res, 1996. **11**(7): p. 1010-8.

35. Mosekilde, L., *Sex differences in age-related loss of vertebral trabecular bone mass and structure--biomechanical consequences.* Bone, 1989. **10**(6): p. 425-32.

36. Seeman, E., et al., *Reduced femoral neck bone density in the daughters of women with hip fractures: the role of low peak bone density in the pathogenesis of osteoporosis.* J Bone Miner Res, 1994. **9**(5): p. 739-43.

37. Van Pottelbergh, I., et al., *Deficient acquisition of bone during maturation underlies idiopathic osteoporosis in men: evidence from a three-generation family study.* J Bone Miner Res, 2003. **18**(2): p. 303-11.

38. Hedstrom, M., *Hip fracture patients, a group of frail elderly people with low bone mineral density, muscle mass and IGF-I levels.* Acta Physiol Scand, 1999. **167**(4): p. 347-50.

39. Lissett, C.A. and S.M. Shalet, *Effects of growth hormone on bone and muscle.* Growth Horm IGF Res, 2000. **10 Suppl B**: p. S95-101.

40. Rosen, T., et al., *Increased fracture frequency in adult patients with hypopituitarism and GH deficiency.* Eur J Endocrinol, 1997. **137**(3): p. 240-5.

41. Ohlsson, C., et al., *Growth hormone and bone.* Endocr Rev, 1998. **19**(1): p. 55-79.

42. Rudman, D., et al., *Effects of human growth hormone in men over 60 years old.* N Engl J Med, 1990. **323**(1): p. 1-6.

43. Martini, G., et al., *Influence of insulin-like growth factor-1 and leptin on bone mass in healthy postmenopausal women.* Bone, 2001. **28**(1): p. 113-7.

44. Sugimoto, T., et al., *Serum levels of insulin-like growth factor (IGF) I, IGF-binding protein (IGFBP)-2, and IGFBP-3 in osteoporotic patients with and without spinal fractures.* J Bone Miner Res, 1997. **12**(8): p. 1272-9.

45. Johansson, A.G., et al., *Growth hormone-dependent insulin-like growth factor binding protein is a major determinant of bone mineral density in healthy men.* J Bone Miner Res, 1994. **9**(6): p. 915-21.

46. S. Høidrup, A.J., S. Schifter, N. E. Skakkebæk, A. N. Pedersen, J. B. Lauritzen and M. Schroll, *Serum insulin-like growth factor (IGF)-I and IGF binding protein-3 correlate with bone mineral density in 80-year-old males but not in females* Osteoporosis International, 1996. **6**(1).

47. Poehlman, E.T., et al., *Menopause-associated changes in plasma lipids, insulin-like growth factor I and blood pressure: a longitudinal study.* Eur J Clin Invest, 1997. **27**(4): p. 322-6.

48. Romagnoli, E., et al., *Effect of estrogen deficiency on IGF-I plasma levels: relationship with bone mineral density in perimenopausal women.* Calcif Tissue Int, 1993. **53**(1): p. 1-6.

49. Cao, J.J., *Effects of obesity on bone metabolism.* J Orthop Surg Res, 2011. **6**: p. 30.

50. Zhao, L.J., et al., *Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis.* J Bone Miner Res, 2008. **23**(1): p. 17-29.

51. Thomas, T.B., B. , *Is Leptin the Link Between Fat and Bone Mass?* Journal of Bone and Mineral Research, 2002. **17**: p. 1563-1569.

52. Hamrick, M.W., et al., *Leptin deficiency produces contrasting phenotypes in bones of the limb and spine.* Bone, 2004. **34**(3): p. 376-83.

53. Agbaht, K., et al., *Circulating adiponectin represents a biomarker of the association between adiposity and bone mineral density.* Endocrine, 2009. **35**(3): p. 371-9.

54. Zoico, E.Z., M.; Di Francesco, V.; Mazzali, G.; Fantin, F.; De Pergola, G.; Zivelonghi, A.; Adami, S.; Bosello, O. , *Relation between adiponectin and bone mineral density in elderly post-menopausal women: role of body composition, leptin, insulin resistance, and dehydroepiandrosterone sulfate.* Journal of Endocrinology Investment, 2008. **31**(4): p. 297-302.

55. Jurimae, J., et al., *The influence of ghrelin, adiponectin, and leptin on bone mineral density in healthy postmenopausal women.* J Bone Miner Metab, 2008. **26**(6): p. 618-23.

56. Center, J.R., et al., *Hormonal and biochemical parameters in the determination of osteoporosis in elderly men.* J Clin Endocrinol Metab, 1999. **84**(10): p. 3626-35.

57. Ho-Pham, L.T., et al., *Contributions of lean mass and fat mass to bone mineral density: a study in postmenopausal women.* BMC Musculoskelet Disord, 2010. **11**: p. 59.

58. Li, S., et al., *Relationship between soft tissue body composition and bone mass in perimenopausal women.* Maturitas, 2004. **47**(2): p. 99-105.

59. Madsen, K.L., W.C. Adams, and M.D. Van Loan, *Effects of physical activity, body weight and composition, and muscular strength on bone density in young women.* Med Sci Sports Exerc, 1998. **30**(1): p. 114-20.

60. Winters, K.M. and C.M. Snow, *Body composition predicts bone mineral density and balance in premenopausal women.* J Womens Health Gend Based Med, 2000. **9**(8): p. 865-72.

61. Witzke, K.A. and C.M. Snow, *Lean body mass and leg power best predict bone mineral density in adolescent girls.* Med Sci Sports Exerc, 1999. **31**(11): p. 1558-63.

62. Bakhireva, L.N., et al., *Modifiable predictors of bone loss in older men: a prospective study.* Am J Prev Med, 2004. **26**(5): p. 436-42.

63. Rector, R.S., et al., *Participation in road cycling vs running is associated with lower bone mineral density in men.* Metabolism, 2008. **57**(2): p. 226-32.

64. Dook, J.E., et al., *Exercise and bone mineral density in mature female athletes.* Med Sci Sports Exerc, 1997. **29**(3): p. 291-6.

65. Duncan, C.S., et al., *Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength.* Med Sci Sports Exerc, 2002. **34**(2): p. 286-94.

66. Fehling, P.C., et al., *A comparison of bone mineral densities among female athletes in impact loading and active loading sports.* Bone, 1995. **17**(3): p. 205-10.

67. Reid, I.R., M.C. Evans, and R.W. Ames, *Volumetric bone density of the lumbar spine is related to fat mass but not lean mass in normal postmenopausal women.* Osteoporos Int, 1994. **4**(6): p. 362-7.

68. Ryan, A.S., et al., *Regional bone mineral density after resistive training in young and older men and women.* Scand J Med Sci Sports, 2004. **14**(1): p. 16-23.

69. Manore, M.M., L.C. Kam, and A.B. Loucks, *The female athlete triad: components, nutrition issues, and health consequences.* J Sports Sci, 2007. **25 Suppl 1**: p. S61-71.

70. Ihle, R. and A.B. Loucks, *Dose-response relationships between energy availability and bone turnover in young exercising women.* J Bone Miner Res, 2004. **19**(8): p. 1231-40.

71. Goulding, A., et al., *Children who avoid drinking cow's milk are at increased risk for prepubertal bone fractures.* J Am Diet Assoc, 2004. **104**(2): p. 250-3.

72. Lukert, B., J. Higgins, and M. Stoskopf, *Menopausal bone loss is partially regulated by dietary intake of vitamin D.* Calcif Tissue Int, 1992. **51**(3): p. 173-9.

73. Cummings, S.R., et al., *Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group.* N Engl J Med, 1998. **339**(11): p. 733-8.

74. Whiting, S.J., et al., *Factors that affect bone mineral accrual in the adolescent growth spurt.* J Nutr, 2004. **134**(3): p. 696S-700S.

75. Gao, X., et al., *Meeting adequate intake for dietary calcium without dairy foods in adolescents aged 9 to 18 years (National Health and Nutrition Examination Survey 2001-2002).* J Am Diet Assoc, 2006. **106**(11): p. 1759-65.

76. Rockell, J.E., et al., *Two-year changes in bone and body composition in young children with a history of prolonged milk avoidance.* Osteoporos Int, 2005. **16**(9): p. 1016-23.

77. Matkovic, V., et al., *Urinary calcium, sodium, and bone mass of young females.* Am J Clin Nutr, 1995. **62**(2): p. 417-25.

78. de Vernejoul, M.C., et al., *Evidence for defective osteoblastic function. A role for alcohol and tobacco consumption in osteoporosis in middle-aged men.* Clin Orthop Relat Res, 1983(179): p. 107-15.

79. Chappard, D., et al., *Alcoholic cirrhosis and osteoporosis in men: a light and scanning electron microscopy study.* J Stud Alcohol, 1991. **52**(3): p. 269-74.

80. Hogan, H.A., et al., *Alcohol consumption by young actively growing rats: a study of cortical bone histomorphometry and mechanical properties.* Alcohol Clin Exp Res, 1997. **21**(5): p. 809-16.

81. Bikle, D.D., et al., *Alcohol-induced bone disease: relationship to age and parathyroid hormone levels.* Alcohol Clin Exp Res, 1993. **17**(3): p. 690-5.

82. Krawitt, E.L., *Effect of ethanol ingestion on duodenal calcium transport.* J Lab Clin Med, 1975. **85**(4): p. 665-71.

83. Diaz, M.N., T.W. O'Neill, and A.J. Silman, *The influence of family history of hip fracture on the risk of vertebral deformity in men and women: the European Vertebral Osteoporosis Study.* Bone, 1997. **20**(2): p. 145-9.

84. Law, M.R. and A.K. Hackshaw, *A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect.* BMJ, 1997. **315**(7112): p. 841-6.

85. Riebel, G.D., et al., *The effect of nicotine on incorporation of cancellous bone graft in an animal model.* Spine (Phila Pa 1976), 1995. **20**(20): p. 2198-202.

86. Boot, A.M., et al., *Bone mineral density and nutritional status in children with chronic inflammatory bowel disease.* Gut, 1998. **42**(2): p. 188-94.

87. Misra, M., et al., *Bone metabolism in adolescent boys with anorexia nervosa.* J Clin Endocrinol Metab, 2008. **93**(8): p. 3029-36.

88. Mora, S., et al., *A prospective, longitudinal study of the long-term effect of treatment on bone density in children with celiac disease.* J Pediatr, 2001. **139**(4): p. 516-21.

89. Zipfel, S., et al., *Osteoporosis in eating disorders: a follow-up study of patients with anorexia and bulimia nervosa.* J Clin Endocrinol Metab, 2001. **86**(11): p. 5227-33.

90. Karsenty, G., *The central regulation of bone remodeling.* Trends Endocrinol Metab, 2000. **11**(10): p. 437-9.

91. Haney, E.M., S.J. Warden, and M.M. Bliziotes, *Effects of selective serotonin reuptake inhibitors on bone health in adults: time for recommendations about screening, prevention and management?* Bone, 2010. **46**(1): p. 13-7.

92. Pan, G., et al., *Pathogenesis of osteopenia/osteoporosis induced by highly active anti-retroviral therapy for AIDS.* Ann N Y Acad Sci, 2006. **1068**: p. 297-308.

93. Dugdale, D.C., *Celiac disease-sprue* in *A.D.A.M. Medical Encyclopedia*. 2010, Merriam-Webster, Incorporated: Springfield, MA.

94. Lakatos, P., *Thyroid hormones: beneficial or deleterious for bone?* Calcif Tissue Int, 2003. **73**(3): p. 205-9.

95. Lewiecki, E.M., *Management of skeletal health in patients with asymptomatic primary hyperparathyroidism.* J Clin Densitom, 2010. **13**(4): p. 324-34.

96. Yeh, H.S. and J.R. Berenson, *Treatment for myeloma bone disease.* Clin Cancer Res, 2006. **12**(20 Pt 2): p. 6279s-6284s.

97. T.P. Van Staa, H.G.L., L. Abenhaim, B. Zhang, C. Cooper, *Use of Corticosteriods and risk of fractures.* J Bone Miner Res, 2000. **15**: p. 993-1000.

98. VanderWalde, A. and A. Hurria, *Aging and osteoporosis in breast and prostate cancer.* CA Cancer J Clin, 2011. **61**(3): p. 139-56.

99. Cameron, D.A., et al., *Bone mineral density loss during adjuvant chemotherapy in pre-menopausal women with early breast cancer: is it dependent on oestrogen deficiency?* Breast Cancer Res Treat, 2010. **123**(3): p. 805-14.

100. Holmes, S.J., et al., *Reduced bone mineral density in men following chemotherapy for Hodgkin's disease.* Br J Cancer, 1994. **70**(2): p. 371-5.

101. Shuster, L.T., et al., *Premature menopause or early menopause: long-term health consequences.* Maturitas, 2010. **65**(2): p. 161-6.

102. Ramaswamy, B. and C.L. Shapiro, *Osteopenia and osteoporosis in women with breast cancer.* Semin Oncol, 2003. **30**(6): p. 763-75.

103. Richards, J.B., et al., *Effect of selective serotonin reuptake inhibitors on the risk of fracture.* Arch Intern Med, 2007. **167**(2): p. 188-94.

104. Hubbard, R., et al., *Exposure to tricyclic and selective serotonin reuptake inhibitor antidepressants and the risk of hip fracture.* Am J Epidemiol, 2003. **158**(1): p. 77-84.

105. Tebas, P., et al., *Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy.* AIDS, 2000. **14**(4): p. F63-7.

106. Greendale, G.A., S. Edelstein, and E. Barrett-Connor, *Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study.* J Bone Miner Res, 1997. **12**(11): p. 1833-43.

107. Gennari, L., R. Nuti, and J.P. Bilezikian, *Aromatase activity and bone homeostasis in men.* J Clin Endocrinol Metab, 2004. **89**(12): p. 5898-907.

108. van Geel, T.A., et al., *Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study.* Eur J Endocrinol, 2009. **160**(4): p. 681-7.

109. van den Beld, A.W., et al., *Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men.* J Clin Endocrinol Metab, 2000. **85**(9): p. 3276-82.

110. Chen, J., J. Kim, and J.T. Dalton, *Discovery and therapeutic promise of selective androgen receptor modulators.* Mol Interv, 2005. **5**(3): p. 173-88.

111. Stone, K., et al., *Hormonal predictors of bone loss in elderly women: a prospective study. The Study of Osteoporotic Fractures Research Group.* J Bone Miner Res, 1998. **13**(7): p. 1167-74.

112. van Hemert, A.M., et al., *Sex hormone binding globulin in postmenopausal women: a predictor of osteoporosis superior to endogenous oestrogens.* Clin Endocrinol (Oxf), 1989. **31**(4): p. 499-509.

113. Johnston, C.C., Jr., et al., *Early menopausal changes in bone mass and sex steroids.* J Clin Endocrinol Metab, 1985. **61**(5): p. 905-11.

114. Slemenda, C., et al., *Sex steroids, bone mass, and bone loss. A prospective study of pre-, peri-, and postmenopausal women.* J Clin Invest, 1996. **97**(1): p. 14-21.

115. Drinka, P.J., et al., *Lack of association between free testosterone and bone density separate from age in elderly males.* Calcif Tissue Int, 1993. **52**(1): p. 67-9.

116. Murphy, S., et al., *Sex hormones and bone mineral density in elderly men.* Bone Miner, 1993. **20**(2): p. 133-40.

117. Oz, O.K., et al., *Bone phenotype of the aromatase deficient mouse.* J Steroid Biochem Mol Biol, 2001. **79**(1-5): p. 49-59.

118. Miyaura, C., et al., *Sex- and age-related response to aromatase deficiency in bone.* Biochem Biophys Res Commun, 2001. **280**(4): p. 1062-8.

119. Hakkinen, K. and A. Pakarinen, *Muscle strength and serum testosterone, cortisol and SHBG concentrations in middle-aged and elderly men and women.* Acta Physiol Scand, 1993. **148**(2): p. 199-207.

120. Gower, B.A. and L. Nyman, *Associations among oral estrogen use, free testosterone concentration, and lean body mass among postmenopausal women.* J Clin Endocrinol Metab, 2000. **85**(12): p. 4476-80.

121. Khosla, S., et al., *Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population-based study.* J Clin Endocrinol Metab, 1997. **82**(5): p. 1522-7.

122. Khosla, S., et al., *Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men.* J Clin Endocrinol Metab, 2001. **86**(8): p. 3555-61.

123. Gu, G., et al., *Estrogen protects primary osteocytes against glucocorticoid-induced apoptosis.* Apoptosis, 2005. **10**(3): p. 583-95.

124. Meema, S., M.L. Bunker, and H.E. Meema, *Preventive effect of estrogen on postmenopausal bone loss.* Arch Intern Med, 1975. **135**(11): p. 1436-40.

125. Weiss, N.S., et al., *Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen.* N Engl J Med, 1980. **303**(21): p. 1195-8.

126. Anderson, F.H., et al., *Androgen supplementation in eugonadal men with osteoporosis: effects of six months' treatment on markers of bone formation and resorption.* J Bone Miner Res, 1997. **12**(3): p. 472-8.

127. Dunstan, C.R., et al., *Bone death in hip fracture in the elderly.* Calcif Tissue Int, 1990. **47**(5): p. 270-5.

128. Tomkinson, A., et al., *The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone.* J Clin Endocrinol Metab, 1997. **82**(9): p. 3128-35.

129. Creighton, D.L., et al., *Weight-bearing exercise and markers of bone turnover in female athletes.* J Appl Physiol, 2001. **90**(2): p. 565-70.

130. Nichols, J.F., J.E. Palmer, and S.S. Levy, *Low bone mineral density in highly trained male master cyclists.* Osteoporos Int, 2003. **14**(8): p. 644-9.

131. Turner, C.H., *Three rules for bone adaptation to mechanical stimuli.* Bone, 1998. **23**(5): p. 399-407.

132. Frost, H.M., ed. *The Laws of Bone Structure* 1964, Thomas: Springfield.

133. Rubin, C.T. and K.J. McLeod, *Promotion of bony ingrowth by frequency-specific, low-amplitude mechanical strain.* Clin Orthop Relat Res, 1994(298): p. 165-74.

134. Robling, A.G., D.B. Burr, and C.H. Turner, *Partitioning a daily mechanical stimulus into discrete loading bouts improves the osteogenic response to loading.* J Bone Miner Res, 2000. **15**(8): p. 1596-602.

135. Lanyon, L.E. and S. Bourn, *The influence of mechanical function on the development and remodeling of the tibia. An experimental study in sheep.* J Bone Joint Surg Am, 1979. **61**(2): p. 263-73.

136. Owan, I., et al., *Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain.* Am J Physiol, 1997. **273**(3 Pt 1): p. C810-5.

137. Robling, A.G., *Is bone's response to mechanical signals dominated by muscle forces?* Med Sci Sports Exerc, 2009. **41**(11): p. 2044-9.

138. Turner, C.H., M.R. Forwood, and M.W. Otter, *Mechanotransduction in bone: do bone cells act as sensors of fluid flow?* FASEB J, 1994. **8**(11): p. 875-8.

139. Duncan, R.L. and C.H. Turner, *Mechanotransduction and the functional response of bone to mechanical strain.* Calcif Tissue Int, 1995. **57**(5): p. 344-58.

140. Reich, K.M., C.V. Gay, and J.A. Frangos, *Fluid shear stress as a mediator of osteoblast cyclic adenosine monophosphate production.* J Cell Physiol, 1990. **143**(1): p. 100-4.

141. Judex, S. and K.J. Carlson, *Is bone's response to mechanical signals dominated by gravitational loading?* Med Sci Sports Exerc, 2009. **41**(11): p. 2037-43.

142. Kohrt, W.M., D.W. Barry, and R.S. Schwartz, *Muscle forces or gravity: what predominates mechanical loading on bone?* Med Sci Sports Exerc, 2009. **41**(11): p. 2050-5.

143. Stewart, A.D. and J. Hannan, *Total and regional bone density in male runners, cyclists, and controls.* Med Sci Sports Exerc, 2000. **32**(8): p. 1373-7.

144. Sabo, D., et al., *Bone quality in the lumbar spine in high-performance athletes.* Eur Spine J, 1996. **5**(4): p. 258-63.

145. Warner, S.E., J.M. Shaw, and G.P. Dalsky, *Bone mineral density of competitive male mountain and road cyclists.* Bone, 2002. **30**(1): p. 281-6.

146. Hetland, M.L., J. Haarbo, and C. Christiansen, *Low bone mass and high bone turnover in male long distance runners.* J Clin Endocrinol Metab, 1993. **77**(3): p. 770-5.

147. Heinonen, A., et al., *Bone mineral density of female athletes in different sports.* Bone Miner, 1993. **23**(1): p. 1-14.

148. Rico, H., et al., *Bone mineral content and body composition in postpubertal cyclist boys.* Bone, 1993. **14**(2): p. 93-5.

149. Rector, R.S., et al., *Lean body mass and weight-bearing activity in the prediction of bone mineral density in physically active men.* J Strength Cond Res, 2009. **23**(2): p. 427-35.

150. Heinonen, A., et al., *Effects of unilateral strength training and detraining on bone mineral mass and estimated mechanical characteristics of the upper limb bones in young women.* J Bone Miner Res, 1996. **11**(4): p. 490-501.

151. Daly, R.M., et al., *The relationship between muscle size and bone geometry during growth and in response to exercise.* Bone, 2004. **34**(2): p. 281-7.

152. Proctor, K.L., et al., *Upper-limb bone mineral density of female collegiate gymnasts versus controls.* Med Sci Sports Exerc, 2002. **34**(11): p. 1830-5.

153. Haapasalo, H., et al., *Dimensions and estimated mechanical characteristics of the humerus after long-term tennis loading.* J Bone Miner Res, 1996. **11**(6): p. 864-72.

154. Kannus, P., et al., *The site-specific effects of long-term unilateral activity on bone mineral density and content.* Bone, 1994. **15**(3): p. 279-84.

155. Vuori, I., et al., *Effects of unilateral strength training and detraining on bone mineral density and content in young women: a study of mechanical loading and deloading on human bones.* Calcif Tissue Int, 1994. **55**(1): p. 59-67.

156. Lang, T., et al., *Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight.* J Bone Miner Res, 2004. **19**(6): p. 1006-12.

APPENDIX A—Study Flyer

WEIGHT BEARING EXERCISE AND BONE HEALTH

The Department of Nutrition and Exercise Physiology is seeking individuals for a study on how different types of weight-bearing exercise affect bone health.

We are looking for:

- Healthy men, 25-60 years old
- Currently participating in 4hr/wk of physical activity for the last 2 years
- Not currently participating in strength training (weight- lifting) or plyometrics (high-intensity jumping)

You will receive:

- free and supervised weight-lifting or plyometric training
- body composition testing
- bone mineral density assessment
- $1000 compensation after completing the study

Contact: N & EP Bone Study
Dept. of Nutrition and Exercise Physiology
106 McKee Gym, 882-9917
Email: mubonestudy@gmail.com

APPENDIX B—Informed Consent

CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

INVESTIGATOR'S NAME: **PAMELA S. HINTON PH.D.**

PROJECT # 1095877

DATE OF PROJECT APPROVAL: SEPTEMBER 12, 2007

FOR HS IRB USE ONLY
APPROVED _____ HS IRB Authorized Representative Date **EXPIRATION DATE:** _____

STUDY TITLE: EFFICACY OF PLYOMETRICS TO INCREASE BONE MASS IN MALES WITH LOW BONE MINERAL DENSITY

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.

This is a research study. Research studies include only people who choose to participate. As a study participant you have the right to know about the procedures that will be used in this research study so that you can make the

decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You are being asked to take part in this study because you are a healthy male who participates in leisure time physical activity.

This study is being sponsored by the Department of Nutrition and Exercise Physiology, University of Missouri-Columbia.

In order to participate in this study, it will be necessary to give your written consent.

WHY IS THIS STUDY BEING DONE?

The purpose of this research is to determine how effective long term (12 months) jump training (plyometrics) is at improving bone density and increasing hormones that promote bone formation, as compared to long-term resistance training. This research is being done because the long-term benefits of regular plyometric exercise or resistance training on bone health in males with below normal bone density are unclear.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 250 people will take part in this study at the University of Missouri-Columbia.

WHAT IS INVOLVED IN THE STUDY?

Visit 1: Begin the informed consent process and describe the study purpose and the requirements.

All participants must: 1) be males between 25 and 60 years of age who participate exclusively in leisure time physical activity at least 4 hours per week for the past 24 months; 2) be apparently healthy; 3) be physically able to perform plyometrics or

resistance training; 4) be willing to keep daily records of physical activity and food intake; 5) be willing and able to provide accurate information about your medical history; 6) follow a normal sleep/wake cycle; and 7) be willing to take a calcium and vitamin D supplement daily.

All participants must not: 1) smoke, or have quit smoking within the last 6 months; 2) drink excessive amounts of alcohol (more than 3 drinks per day); 3) take medication that affects bone; 4) have a disease that affects bone; or 5) participate regularly in plyometrics or resistance training.

Visit 2: If you decide to participate in the study you will come back for Visit 2 and sign the consent form. Then you will undergo a dual X-ray absorptiometry (DXA) bone density test. You will be required to lie still for approximately 10 minutes during this procedure. You will be exposed to a small amount of radiation during the scan, equivalent to $1/10^{th}$ the radiation of a chest X-ray and about 1/1000 of a similar Computed Tomography scan. All study participants will undergo additional bone density tests at 6 and 12 months. **It is important to note that in order to be eligible to participate in this study, the DXA scan must indicate that you have below normal bone mineral density.** You will be provided with the results of your bone mineral density test. If you have any questions about the results you will need to contact your family practitioner. Interpretation of the results of your bone mineral density test must be performed by a physician.

You will also fill out a medical and physical activity history questionnaire. You must provide information about your medical history, including history of illness, injuries, and drug treatment that may affect your ability to safely and effectively participate in the study. You also must provide accurate information about your physical activity history.

If you meet the eligibility requirements of the study (i.e., age, activity level, no diseases or medications that affect bone, below normal bone mineral density), you will be provided a 7-Day diet record form to record your dietary intake and return at the next visit. You will also be given a form to record your physical training for 7 days.

Visit 3: You will have your blood drawn on five occasions during the study (0, 3, 6, 9, 12 months). Following an 8-12 hour fast, your height and weight will be measured and a blood sample will be taken from a vein in your forearm using the same procedure as would be followed at a health clinic. On three of these occasions (0, 6, and 12 months)

additional blood samples (3) will be collected during the 24 hours after your normally scheduled training. The amount of the blood sample is very small and will not affect your health (15 mL, 1 tablespoon). The blood will be used to measure markers of bone formation and breakdown and hormone levels. Your blood will be analyzed for factors that may affect your bone mass.

Your blood will be kept frozen for 5 years after the study is completed and the results are published in a research journal. No additional tests will be performed on your blood sample.

The study will require regular visits to the Exercise Physiology Laboratory, each visit lasting 30-90 minutes during the course of the exercise intervention. On several occasions (0, 6, and 12 months) during your normally scheduled training we will determine your feelings of pain, fatigue and exertion using surveys to help determine your experience with the training program and monitor your risk for pain and/or injury.

You will continue your normal exercise program throughout the study and you will maintain your normal life at home, work or school. You are allowed to quit at any time without penalty or loss of any benefits. You will be asked to discontinue the study if the research and medical staff determine it is in your best interest to do so.

You will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. Neither you nor the researcher will choose what group you will be in. You will have an equal chance of being placed in either group.

Interventions: All exercise training sessions will be conducted at the McKee Gym Fitness Center, under the supervision of trained exercise personnel.

Group 1: If you are participating in the plyometric intervention you will attend 3 training sessions per week until you complete the 12-month exercise intervention.

Participants will complete 10 repetitions of 10 different exercises to accumulate 40-120 loading cycles (jumps). The plyometric exercise sets will include: squat jumps, forward

hops, split squat jumps, , lateral box push offs, bounding, bounding with rings (lateral), box drill with rings, lateral hurdle jumps, zigzag hops, single leg lateral hops, and progressive depth jumps (10-100cm). The intensity of plyometric training will progress, with low intensity jumps weeks 1-2, low and moderate jumps weeks 3-4, and high intensity jumps weeks 5-6, followed by a rest week. You will steadily increase the intensity and number of jumps over each training cycle.

Group 2: If you are participating in the resistance training intervention you will attend 2training sessions per week until you complete the 12-month exercise intervention.

Each exercise session will be made up from the following resistance exercises: squats, bent over row, dead lift, military press, lunges, and calf raises. Prior to and every 6 weeks during the RET intervention, maximal strength testing will be performed. This will involve a warm-up set of 5-10 repetitions, equal to 40-60% of your perceived maximum for each exercise. After a brief rest period, a second set of 3-5 repetitions at an intensity between 60-80% of perceived maximum will be performed. Subsequent attempts will be conducted using incremental increases in weight until a failed attempt, typically within 3 to 5 maximal attempts. One repetition maximums (1RM) will be conducted for squat, dead lift, and military press exercises, and modified maximums (10 repetitions) will be calculated for exercises in which 1RM are not commonly performed.

To account for strength adaptations as a result of strength training improvements, a progressive exercise program will be used. Weeks 1-2 will include one warm-up set (10 repetitions at 20% 1RM) and 3 moderate intensity sets (10 repetitions at 50% 1RM) for each exercise performed. Weeks 3-4 will be comprised of one warm-up set (10 repetitions at 20% 1RM), two sets at a moderate intensity (10 repetitions at 60% 1RM), and one set at high intensity (6-8 repetitions at 70-75% 1RM). Weeks 5-6 will be comprised of one warm-up set (10 repetitions at 20% 1RM), two sets at moderate intensity (10 repetitions at 60% 1RM), and one set at high intensity (3-5 repetitions at 80-90% 1RM). Week 7 will be a rest week.

HOW LONG WILL I BE IN THE STUDY?

Completion of all exercise training and testing procedures will take approximately 12 months.

You can stop participating at any time. Your decision to withdraw from the study will not affect in any way your medical care and/or benefits.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your doctor. There may also be other side effects that we cannot predict.

Risks and side effects related to the study tests and procedures include:

There is a possibility of bruising and soreness at the site of the blood draw. Sterile procedures will be used so the chance of getting an infection is very remote.

There is a possibility of muscle and joint injury as a result of participating in the weight lifting exercises of the resistance training and the jumping of the plyometric training. Participants will be instructed in the safe and proper procedures for all exercise activities by qualified exercise physiologists and supervised by exercise personnel at all times. All exercise sessions will include warm-up and cool-down procedures to further minimize the risk of injury.

Reproductive risks: The effects of the DXA scan on the male reproductive system are unknown but could cause harm. If you have any questions about the reproductive issues, please discuss them with the investigator or your doctor.

You will be exposed to a small amount of radiation. Radiation effects are cumulative. You should always inform future doctors of your participation in this study.

For the reasons stated above the investigator will observe you closely during the study described above and, if you have any worrisome symptoms, notify the investigator immediately. Dr. Pam Hinton's telephone number is (573) 882-4137. For more information about risks and side effects, ask the investigator or contact Dr. Hinton at (573) 882-4137.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. You may expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge. We hope the information learned from this study will allow for more specific exercise prescriptions for men with low bone mineral density.

In addition, you will: 1) participate in a supervised exercise program; 2) potentially improve your bone mass, strength, and balance; 3) receive free bone mineral density screening and results; 4) receive free diet and physical activity analyses; 5) receive free calcium and vitamin D supplements; and 5) have free parking and access to the McKee Gym locker room and showers during exercise sessions.

WHAT OTHER OPTIONS ARE THERE?

You have the option to not participate in this study.

WHAT ABOUT CONFIDENTIALITY?

Information will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study personnel at the University of Missouri-Columbia in a form that could identify you without your written consent, except as required by law. If the investigator conducting this study is not your primary, or regular doctor, she must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in this study.

It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the study sponsor (and/or its agent), the Food and Drug Administration (FDA), federal or state government agencies, University of Missouri Health Sciences Institutional Review Board or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Missouri-Columbia will use reasonable efforts to protect your privacy and the confidentiality of your medical information.

The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will

not be used in any publication or teaching materials without your specific permission.

WHAT ARE THE COSTS?

There is no cost to you for the study procedures. You will not be charged for blood tests that are part of this research study.

WILL I BE PAID FOR PARTICIPATING IN THE STUDY?

You will be compensated $1000 for completion of the study. You will be paid $300 for completion of the first six months of the study and an additional $700 upon completion of the entire study.

WHAT IF I AM INJURED?

It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Participation in this study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate. If you decide to participate, you can change your mind and drop out of the study at any time without affecting your present or future care in the University of Missouri-Columbia. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. In addition, the investigator of this study may decide to end your participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed.

You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Dr. Pam Hinton at (573) 882-4137 or Dr. John Thyfault at (573) 882-9818.

A copy of this consent form will be given to you to keep.

SIGNATURE

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

Subject/Patient*	Date
Legal Guardian/Advocate/Witness (if required)**	Date
Additional Signature (if required) (identify relationship to subject)***	Date

*A minor's signature on this line indicates his/her assent to participate in this study. A minor's signature is not required if he/she is under 7 years old. Use the "Legal Guardian/Advocate/Witness" line for the parent's signature, and you may use the "Additional Signature" line for the second parent's signature, if required.

**The presence and signature of an impartial witness is required during the entire informed consent discussion if the patient or patient's legally authorized representative is unable to read.

***The "Additional Signature" line may be used for the second parent's signature, if required. This line may also be used for any other signature which is required as per federal, state, local, sponsor and/or any other entity requirements.

"If required" means that the signature line is signed only if it is required as per federal, state, local, sponsor and/or any other entity requirements.

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

Study Representative****	Date

****Study Representative is a person authorized to obtain consent. Per the policies of the University of Missouri Health Care, for any 'significant risk/treatment' study, the Study Representative must be a physician who is either the Principal or Co-Investigator. If the study is deemed either 'significant risk/non-treatment' or 'minimal risk,' the Study Representative may be a non-physician study investigator.

APPENDIX C—HIPPA

UNIVERSITY OF MISSOURI-COLUMBIA
Institutional Review Board

HIPAA AUTHORIZATION FORM

Authorization for the Use and Disclosure of Personal Health Information Resulting from Participation in a Research Study

FOR IRB USE ONLY	
Acknowledged	
IRB Authorized Representative	Date 3/12/09

Principal Investigator's Name: Dr Hinton
Project # 1095877
Project Title: Efficacy of Plyometrics to Increase Bone Mass in Male Cyclists with Osteopenia

You have agreed to participate in the study mentioned above. This authorization form gives more detailed information about how your health information will be protected.

1. Description of the information
My authorization applies to the information described below. Only this information may be used and/or disclosed in accordance with this authorization:
Demographic and anthropomorphic information, results of bone density scan, blood tests, and medical history

2. Who may use and/or disclose the information
I authorize the following persons (or class of persons) to make the authorized use and disclosure of my PHI:
Principal investigator and graduate students research assistants.

3. Who may receive the information
I authorize the following persons (or class of persons) to receive my personal health information
University Missouri IRB, publications, research collaborators, and current and potential funding agencies

4. Purpose of the use or disclosure
My PHI will be used and/or disclosed upon request for the following purposes:
Publications and presentation that will not identify me, auditing, administrative and billing reviews, study outcomes including safety and efficacy

5. Expiration date or event
This authorization expires upon:
☐ The following date: _____
☐ End of research study
☒ No expiration date
☐ Other: _____

6. Right to revoke authorization
I understand that I have a right to revoke this authorization at any time. My revocation must be in writing in a letter sent to the Principal Investigator at 106 McKee Gym, University of Missouri, Columbia, MO 65211. I am

- aware that my revocation is not effective to the extent that the persons I have authorized to use and/or disclose my PHI have already acted in reliance upon this authorization.

7. Statement that re-disclosures are no longer protected by the HIPAA Privacy Rule
I understand that my personal health information will only be used as described in this authorization in relation to the research study. I am also aware that if I choose to share the information defined in this authorization to anyone not directly related to this research project, the law would no longer protect this information. In addition, I understand that if my personal health information is disclosed to someone who is not required to comply with privacy protections under the law, then such information may be re-disclosed and would no longer be protected.

8. Right to refuse to sign authorization and ability to condition treatment, payment, enrollment or eligibility for benefits for research related treatment
I understand that I have a right not to authorize the use and/or disclosure of my personal health information. In such a case I would choose not to sign this authorization document I understand I will not be able to participate in a research study if I do not do so. I also understand that treatment that is part of the research project will be conditioned upon my authorization for the use and/or disclosure of my personal health information to and for use by the research team.

9. Suspension of right to access personal health information
I agree that I will not have a right to access my personal health information obtained or created in the course of the research project until the end of the study.

10. If I have not already received a copy of the University of Missouri Healthcare Privacy Notice, I may request one. If I have any questions or concerns about my privacy rights I should contact, the HS Privacy Officer at 573-882-9054 or the Campus Privacy Officer at 573-882-7254.

11. Individuals' signature and date
I certify that I have received a copy of the authorization.

_____ _____
Signature of Research Participant Date

_____ _____
Research Participant's Legally Authorized Representative Date

_____
Describe Representative Authority to Act for the Participant

APPENDIX D—Medical Questionnaire (PAR-Q)

Physical **A**ctivity **R**eadiness **Q**uestionnaire

Subject number _____

These questions ask about your readiness to participate in this research study's physical activity component. Please read each question carefully and answer each one honestly. Check **YES** or **NO**

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6. Do you have high blood pressure (systolic ≥ 140 mm Hg or diastolic ≥ 90 mm Hg)?
		7. Is your doctor currently prescribing drugs (for example, water pills) for blood pressure or heart condition?
☐	☐	8. Do you know of any other reason why you should not do physical activity?

☐ ☐ 9. Do you have a family history of heart disease (for example, heart attack or sudden death) in first degree relative (male <55 years or female <65 years old)?

☐ ☐ 10. Currently a smoker or quit within previous 6 months?

☐ ☐ 11. Do you have high cholesterol? (Total cholesterol > 200 mg/dl, high-density lipoprotein cholesterol < 35 mg/dl, low-density lipoprotein > 130 mg/dl)

☐ ☐ 12. Do you have impaired fasting glucose? (for example ≥ 110 mg/dl)

_____ 13. During a typical week how many alcoholic beverages do you consume?

_____ 14. What is the greatest number of alcoholic beverages you may consume in a single day?

APPENDIX E—Physical Acitiviy History Questionnaire

Efficacy of Plyometrics to Increase Bone Mass in Men

Medical and Physical Activity History Questionnaire

Subject number _____

Date _____

These questions ask about your medical and physical activity history. Please fill in the blank or circle the appropriate response.

1. Date of Birth: __ __ / __ __ / __ __ __ __
2. Ethnicity: Hispanic or Latino Not Hispanic or Latino
3. Race: African-American/Black

   Alaskan Native

   American Indian

   Asian

   Caucasian/White

   Hawaiian or other Pacific Islander

   Other: _____ (specify)
4. Do you regularly consume soy foods? Yes No
5. Do you currently take a calcium supplement? Yes No

   What dose? _____mg
6. Are you currently taking any medications? Yes No

   If so, which ones_____(specify)
7. Are you <u>currently</u> taking any anti-inflammatory steroids? Yes No

   If so, which ones? _____ (specify)

   How long have you been taking them? _____ (specify)

8. Have you <u>previously</u> taken anti-inflammatory steroids? Yes No

   If so, which ones? _____ (specify)

   When and for how long? _____ (specify)

9. Do you have a family history of osteoporosis? Yes No

   If so, please list affected family members, e.g., maternal grandmother.

   _____

10. Have you ever been diagnosed with a disease that affects bone (Cushing's disease, hyperthyroidism, leukemia, Crohn's disease, chronic liver disease, rheumatoid arthritis, etc)? Yes No

    What was the diagnosis? _____

    When was the diagnosis? _____

    What is your current treatment? _____

11. Have you had any sports related fractures or stress fractures in the past 5 years?

    Fracture: Yes No

    Number _____

    Location on body_____

    _____

    Year_____

    Stress Fracture: Yes No

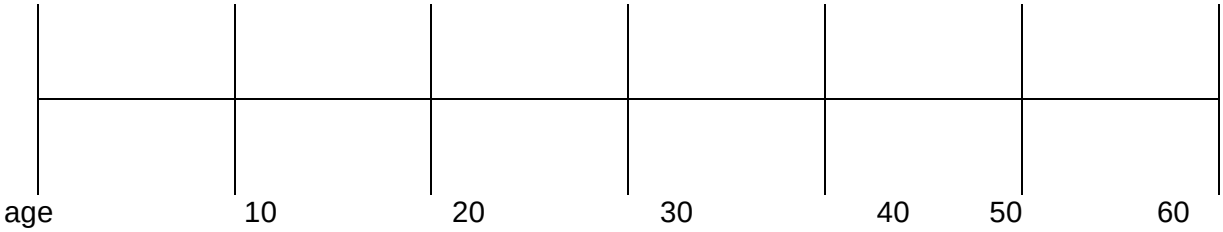
    Number _____

    Location on body_____

_____

Year_____

12. Please use the timeline below to indicate what leisure time physical activity and/or sports (include strength training) you participated in or played during your lifetime.



For each leisure time physical activity and/or sport listed, please describe approximately how many hours per week you participated or competed in this sport. If you competed, please indicate the level of competition.

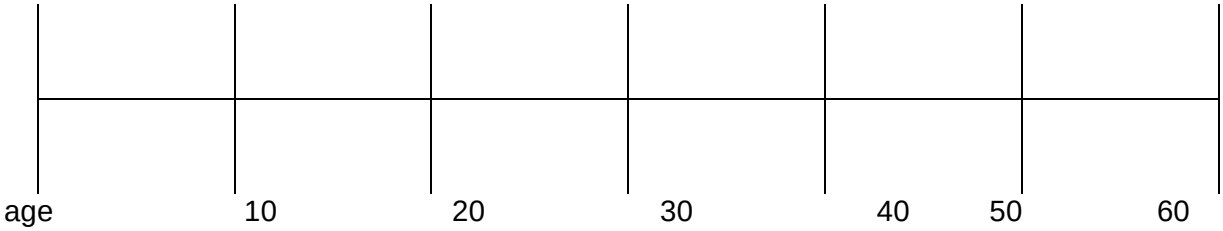
Physical Activity or Sport	Ages	Hours per week	Weeks per year	Level of Competition

13. What leisure time physical activities and/or sports do you participate in now (include strength training)? How many hours per week do you train for or

compete in this leisure time physical activity and/or sport? If you compete, please indicate the level of competition.

Physical Activity or Sport	Hours per week	Weeks per year	Level of Competition

14. Please use the timeline below to indicate job titles and physical activity you have had during your lifetime.



15. During the past 7 days, did you work for pay or as a volunteer (if yes, continue to questions 15-16)? Yes No

16. During the past 7 days, how many hours did you work for pay and/or as a volunteer?
_____ hours.

17. Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

    a. Mainly sitting with slight arm movements. [Examples: office worker, watchmaker, seated assembly line worker, bus driver, etc.]

    b. Sitting or standing with some walking. [Examples: cashier, general office worker, light tool and machinery worker, etc.]

c. Walking with some handling of materials generally weighing less than 50 pounds. [Examples: postal worker, waiter/waitress, construction worker, heavy tool and machinery worker, etc.]

d. Walking and heavy manual work often requiring handling of materials weighing over 50 pounds. [Examples: lumberjack, stone mason, general laborer, etc]

18. Do you have a “normal” sleep pattern i.e., awake during the day, and asleep at night? Yes No

APPENDIX F—Calicum Food Frequency Questionnaire

Food Frequency Questionnaire

INSTRUCTIONS:

Think about **what you typically eat in a normal day,** including breakfast, lunch, dinner, and snacks.

For each item that you eat in the list of foods below, please enter the number of servings you usually eat each day.

Please enter servings in decimals, e.g., 1 serving or 2.5 servings.

HC Foods	Serving Size	Number of Servings
Nonfat or low-fat yogurt	1 cup (8 oz)	
Milk (whole, low-fat or nonfat)	1 cup (8 oz)	
Milkshake (any flavor)	1 cup (8 oz)	
Chocolate milk or hot chocolate (made with whole, low-fat or nonfat)	1 cup (8 oz)	
Cheese (Cheddar/Monterey Jack types)	1-½ oz.	
Processed cheeses (sliced American, string cheese)	1 item	
Soft cheeses (feta, camembert, brie)	1-½ oz.	
Ricotta cheese	½ cup	
Blended coffee drinks (e.g. lattes, mochas, made with milk)	1 - ½ cup	
Lasagna	1 large piece	
Enchilada, cheese	1 large	
Tofu processed with calcium	½ cup (4 oz)	

MLC Foods	Serving Size	Number of Servings
Custard or flan	½ cup (4 oz)	
Pudding	½ cup (4 oz)	
Frozen yogurt	½ cup (4 oz)	
Cottage cheese	1 cup (8 oz)	
Mustard greens, cooked	1 cup (8 oz)	
Bok choy, cooked	1 cup (8 oz)	
Canned fish with bones (salmon, mackerel)	2 oz.	
Parmesan cheese	2 Tbsp.	

Turnip greens, cooked	1 cup (8 oz)	
Kale	1 cup (8 oz)	
Ice milk (full fat, low-fat)	½ cup (4 oz)	
Ice cream	½ cup (4 oz)	
Almonds	¼ cup	
Hot chocolate (made with packet)	1 cup (8 oz)	
Broccoli	1 cup (8 oz)	
Beans, refried beans or peas	1 cup (8 oz)	
Corn tortillas	1 tortilla	
Cream soup	1 cup (8 oz)	
Sardines	1 3-inch sardine	
Cream cheese	1 tablespoon	
Spinach	1 cup (8 oz) fresh	
Macaroni & Cheese	1 cup (8 oz)	

CF Foods	Serving Size	Number of Servings
Calcium-fortified soy beverage	1 cup (8 oz)	
Calcium-fortified orange juice	1 cup (8 oz)	
Calcium-fortified frozen waffles	2 waffles	
Calcium-fortified cereal (100 mg calcium/serving)	1 cup (8 oz)	
Calcium-fortified energy bars	1 bar	

APPENDIX G—7-Day Diet Log

University of Missouri-Columbia Exercise Physiology Lab

Efficacy of Plyometrics to Increase Bone Mass in Men with Osteopenia

7-Day Dietary Record Instructions

In order for us to assess your dietary habits, we need you to log your diet for seven consecutive days. Please be as accurate as possible. **Write down everything you eat, including snacks between meals, and drinks such as coffee, soft drinks, beer, wine and spirits.**

Below are some tips that will help you complete your diet record.

1) Carry the log with you as much as possible.

2) Write down **when** you ate.

3) Be **as specific as possible** about what you ate (e.g. include brand, restaurant), not just the type of food.

4) Write down the **exact amount** you ate using standard household measures (e.g. teaspoon, tablespoon, ounces, cups, number of slices, pieces).

5) Include **food preparation** techniques (e.g. baked, boiled, fried, sautéed, steamed).

6) Include any **condiments** you add (e.g. catsup, croutons, mayonnaise, mustard, onions, pickles).

7) Include any **side items** (e.g. French fries, vegetables, fruits, or salad).

8) Indicate if an item is low-fat, fat-free, etc.

9) Write down **everything** you eat, including snacks, nibbles, gum, and mints.

10) Record all **drinks/beverages**, other than water, **including alcohol**.

11) Record any nutritional and/or vitamin **supplements** (e.g. Powerbar, protein shakes, etc.).

12) Please keep your diet record up to date. Record immediately after each meal or snack. It is

difficult to accurately remember what you ate if you wait until the end of the day to write it down.

Thank you, in advance, for your assistance in making this research study a success!

Please return the records to the lab at your earliest convenience.

ID #: **Session:** **Date:** **Day of week:**

Time of Day	Food/Drink	Brand	Amount (tsp, cup, oz)	Condiments	Location/Place
BREAKFAST					
MORNING SNACK					
LUNCH					
AFTERNOON SNACK					
DINNER					
EVENING SNACK					
SUPPLEMENTS					

APPENDIX H—Physical Activity Log

University of Missouri-Columbia

Exercise Physiology Lab

Efficacy of plyometrics in male cyclists with osteopenia: *Physical Activity Log*

Subject # _____

Week_____ Dates __/__/__-__/__/__

Weight (lbs)_____

Day	Date	Exercise Mode	Total Time (hrs:min)	Distance (miles/yards)	Average Pace (min/mile, mph, yds/min)	Max HR	Avg HR	Intensity (L, M, H)
Mon								
Tues								
Wed								
Thur								
Fri								
Sat								
Sun								

APPENDIX I—Energy Intake and Energy Expenditure.

TABLE 10. Changes in energy intake and energy expenditure from 0 months to 12 months.

	PLY		RT	
	0 Month	12 Month	0 Month	12 Month
Kilocalories (kcal)	2350.14 ± 201.31	2518.47 ± 234.76	2681.66 ± 295.12	2469.62 ± 215.74
Carbohydrates (g)	281.92 ± 30.23	302.95 ± 29.48	339.67 ± 28.80	311.99 ± 43.79
Protein (g)	99.57 ± 9.19	94.01 ± 7.35	107.94 ± 11.15	100.53 ± 8.28
Fat (g)	92.11 ± 8.95	98.91 ± 11.85	97.40 ± 16.89	88.25 ± 8.44
Calcium (mg)	1024.41 ± 142.72	969.20 ± 103.66	1168.65 ± 99.48	770.44 ± 91.09
Vitamin D (IU)	193.59 ± 50.85	134.12 ± 49.40	206.35 ± 66.55	222.89 ± 78.97
PA Expenditure (kcal)	549.51 ± 249.66	312.16 ± 125.05	456.50 ± 137.04	675.02 ± 239.28

There were no significant differences from 0 to 12 months in either group.
Data are means ± S.E.

APPENDIX J—2-Way Bone Mineral Density Repeated Measure ANOVAs.

We ran 2-way repeated measure ANOVAs on all bone mineral density sites using energy expenditure/body weight (kg) at 0 month, 12 month, and the percent change from 0 to 12 month as a covariate.

Whole body bone mineral density had a significant main effect for time, such that whole body bone mineral density increased, when using the percent change of energy expenditure/body weight (kg) as a covariate ($p= 0.038$). Left arm bone mineral density had a significant main effect for time, such that left arm bone mineral density decreased, when using energy expenditure/body weight (kg) at 12 months as a covariate ($p= 0.052$). Hip bone mineral density had a significant main effect for time, such that hip bone mineral density increased, when using energy expenditure/body weight at 0 months as a covariate ($p= 0.036$). Also, hip bone mineral density had a significant interaction effect, such that hip bone mineral density increased in the RT group but maintained in the PLY group, when using energy expenditure/body weight at 12 months as a covariate ($p= 0.019$). There was not a significant change in left leg bone mineral density using energy expenditure/body weight (kg) as a covariate.

TABLE 11. Changes in Bone Mineral Density when using Energy Expenditure/Body Weight as a Covariate.

Covariate	Site	RT		PLY	
EE/BW 0		**0 Month**	**12 Month**	**0 Month**	**12 Month**
	Whole Body	1.153 ± 0.029	1.175 ± 0.031	1.115 ± 0.020	1.121 ±0.022
	Left Arm	0.840 ± 0.023	0.835 ± 0.024	0.788 ± 0.017	0.792 ± 0.017
	Left Leg	1.258 ± 0.049	1.286 ± 0.049	1.229 ± 0.035	1.222 ± 0.035
	Hip*	0.930 ± 0.047	0.938 ± 0.043	0.897 ± 0.033	0.891 ± 0.031
EE/BW 12					
	Whole Body	1.170 ± 0.025	1.194 ± 0.028	1.107 ± 0.023	1.110 ± 0.026
	Left Arm*	0.845 ± 0.022	0.844 ± 0.023	0.790 ± 0.021	0.785 ± 0.021
	Left Leg	1.271 ± 0.038	1.290 ± 0.039	1.211 ± 0.035	1.209 ± 0.036
	Hip**	0.924 ± 0.041	0.942 ± 0.036	0.882 ± 0.036	0.869 ± 0.031
%∆EE/BW					
	Whole Body*	1.145 ± 0.031	1.170 ± 0.034	1.120 ± 0.022	1.122 ± 0.024
	Left Arm	0.834 ± 0.030	0.834 ± 0.028	0.789 ± 0.022	0.788 ± 0.020
	Left Leg	1.238 ± 0.050	1.269 ± 0.051	1.236 ± 0.036	0.228 ± 0.037
	Hip	0.922 ± 0.054	0.934 ± 0.048	0.898 ± 0.039	0.887 ± 0.035

EE/BW 0: energy expenditure/body weight (kg) at 0 months; EE/BW 12: energy expenditure/body weight (kg) at 12 months; %∆EE/BW: percent change of energy expenditure/body weight (kg) from 0 to 12 months.
*Significant main effect for time. **Significant group x time interaction.

APPENDIX K—1-Way Bone Mineral Density Repeated Measure ANOVAs.

We ran 1-way repeated measure ANOVAs on the weight-bearing bone mineral density sites using the percent change of the 1 repetition maximum (1-RM) for squat as a covariate in the RT group. When using the percent change in the 1-RM squat weight, there no significant changes in whole body, left leg, or hip bone mineral density in the RT group.

We also ran 1-way repeated measure ANOVAs on the non-weight-bearing bone mineral density sites using the percent change of the 1-RM for military press as a covariate in the RT group. There were no significant changes.

We ran 1-way repeated measure ANOVAs on all bone mineral density sites using the percent change in vertical jump as a covariate in the PLY group. Hip bone mineral density had a main effect for time, such that hip bone mineral density decreased in the PLY group ($p= 0.015$). There were no significant changes in whole body, left leg, or left arm bone mineral density sites.

TABLE 12. Changes in Bone Mineral Density using Percent Change in 1-Repition Maximums or the Percent Change in Vertical Jump as a Covariate.

Covariate	Site	RT	
%Δ 1-RM Squat		**0 Month**	**12 Month**
	Whole Body	1.180 ± 0.018	1.191 ± 0.021
	Left Leg	1.283 ± 0.043	1.277 ± 0.038
	Hip	0.939 ± 0.021	0.954 ± 0.021
%Δ 1-RM Military Press	Left Arm	0.849 ± 0.020	0.847 ± 0.015

		PLY	
%Δ Vertical Jump		**0 Month**	**12 Month**
	Whole Body	1.115 ± 0.021	1.121 ± 0.024
	Left Arm	0.789 ± 0.016	0.793 ± 0.019
	Left Leg	1.228 ± 0.032	1.223 ± 0.032
	Hip*	0.898 ± 0.037	0.892 ± 0.035

%Δ: percent change; 1-RM: 1 repetition maximum.
*Significant main effect for time.

APPENDIX L—Correlation Coefficients and Significance Values for the Relationships between the Percent Changes in Hormones and the Percent Changes in Lean Body Mass.

TABLE 13. The relationships between the percent change in sex hormones (SHBG, testosterone, free testosterone, and estradiol) and the percent change in lean body mass at the whole body, leg, and arm.

		%Δ Whole Body LBM	%Δ Left Leg LBM	%Δ Left Arm LBM
%Δ SHBG	RT (n=7)	r= -0.312 p= 0.248	r= 0.448 p= 0.157	r= -0.497 p= 0.128
	PLY (n=9)	r= 0.488 p= 0.091	r= 0.480 p= 0.095	r= 0.588* p= 0.048
%Δ Testosterone	RT (n=10)	r= 0.027 p= 0.471	r= -0.448 p= 0.097	r= 0.121 p= 0.370
	PLY (n=8)	r= -0.581 p= 0.065	r= -0.363 p= 0.188	r= 0.233 p= 0.290
%Δ Free Testosterone	RT (n=7)	r= -0.631 p= 0.064	r= 0.559 p= 0.096	r= -0.136 p= 0.386
	PLY (n=6)	r= 0.144 p= 0.393	r= -0.697 p= 0.062	r= 0.678 p= 0.069
%Δ Estradiol	RT (n=8)	r= -0.143 p= 0.367	r= 0.075 p= 0.430	r= -0.014 p= 0.487
	PLY (n=9)	r= -0.182 p= 0.319	r= 0.103 p= 0.396	r= -0.285 p= 0.229

*Significant at the p≤0.050 significance level (one-tailed).
%Δ= percent change, BMD=bone mineral density, LBM=lean body mass.