The Indices of Bone Changes in Response to Exercise

by

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ABSTRACT

The gold standard for bone measurement is DXA (dual energy X-ray absorptiometry). Typically, to observe changes in bone by DXA, a minimum of a 4-month intervention is required. Serum osteocalcin (OST) (a bone formation marker) and quantitative ultrasound (QUS) of the calcaneus can be used as indicators of bone change but the sensitivity and time course of these indices to short term interventions are unknown. The purpose of this study was twofold: to compare monthly changes in OST and QUS in response to jump training and to evaluate the relationship between DXA, OST and QUS. Young women with QUS t-scores less than 1.0 were randomized into a jump training (J) (n=16) or control (C) (n=16). J consisted of a progressive routine of 1 and 2-footed jumping performed 3 days per week for 4 months. Body composition, QUS and OST were measured at baseline, and monthly for 4 months. DXA and 24-hour dietary recalls were completed at baseline and 4 months. Low attrition rate (12.5%) and high compliance (98%) with the exercise intervention was recorded. No significant correlations between QUS and OST existed. No significant differences were observed between groups at baseline in body composition or bone variables. Monthly increases in OST were observed but there were no significant differences over time between groups in any bone variables. OST and QUS may be indicative of short term bone changes but these variables were not specifically sensitive to the jumping intervention in this population of women.

DEDICATION

I dedicate this dissertation to every person who has encouraged me to strive to do the best that I can at everything that I do in life. Without this encouragement from countless people along the way I would not be realizing my childhood goal to teach. I cannot thank my parents enough for their ongoing support. To my Dad, for teaching me right from wrong while also being my friend growing up. To my Mom, for spending time to stay home with me growing up so that I could realize all of my dreams and be successful at school and sports. To my friends, both new and old that have taught me how to be strong, to stand up for what I believe in, and for making me laugh through difficult times. Most of all, I dedicate this culminating work to Chris. I never would have realized my potential to pursue graduate work without his constant encouragement, love, and belief that I could accomplish this when I wanted to quit and didn't believe in myself anymore. I do not think that I would have pursued this degree without his eternal support.

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Introduction

Osteoporosis is a progressive systemic bone disease characterized by low bone mineral density (BMD) and a deterioration of the micro-architecture of bone tissue that compromises bone strength and increases susceptibility to fracture. Bone management is often characterized as a bank savings account where bone growth during youth adds to the bone reserves, but by midlife withdrawals from these savings are greater than deposits. Once withdrawals exceed a critical threshold, then the bone is at risk for fracture. Thus, osteoporosis is considered a pediatric disease with geriatric consequences. Identification and screening of low BMD occurs in late adulthood when the "window" for prevention is gone. According to the World Health Organization (WHO), those with BMD values one standard deviation below the typical bone mass of a young reference value (i.e., t-scores of -1.0) are considered osteopenic. Those with t-scores of -2.5 or below are considered to have osteoporosis. A normal rate of loss is assumed to be approximately 1% per year during pre-menopause and 4% per year during the menopausal stage (WHO, 2004). This increased rate of bone loss is attributed to an estrogen deficiency. It has been estimated that if bone mass t-scores could be elevated one standard deviation, then the risk of fracture would be reduced by 50% at any age (Kriemler, Zahner, Puder, Braun-Fahrlander, Schindler, Farpous-Lambert, Kranzlin, & Rizzoli, 2008). Since peak bone mass attainment occurs between the second and third decade of life, this is the critical period to intervene

(Specker & Schoenau, 2005; Berger, Goltzman, Langsetmo, Joseph, Kreiger, Tenenhouse, Davison, Josse, Prior, Hanley & the CaMos Research Group, 2010).

Clearly, osteoporosis is a serious health threat. For example, up to 24% of those who suffer an osteoporotic fracture will die within one year of sustaining the fracture (Cooper, Atkinson, Jacobsen, O'Fallon, & Melton, 1993; Leibson, Tosteson, Gabriel, Randsom, & Melton, 2002). Minimizing the incidence of osteoporosis through early prevention during childhood remains a major public health goal. Managing bone health requires regular weight bearing exercise and adequate calcium intake within a tightly regulated hormonal milieu. The positive effects of weight-bearing activity on bone are reasonably well-known. However, it is unclear what exercise types, frequencies, or intensities are most effective for maximizing bone growth and strength. For example, daily jump training that was incorporated into the school day in children was shown to increase bone mineral content (BMC) significantly (2-27%) over a 3-8 month period (Gunter, Baxter-Jones, Mirwald, Almstedt, Fuchs, Durski, & Snow, 2008a; Johannsen, Binkley, Englert, Neiderauer, & Specker, 2003; McKay, MacLean, Petit, MackLevie-O'Brien, Janssen, Beck, & Khan, 2005a; Weeks, Young, & Beck, 2008). In young adult women, fairly intense, low-repetition jump training performed three days per week for six months was effective at significantly increasing BMD at the femoral neck and

lumbar spine (Kato, Terashima, Yamashita, Hatanaka, Honda, & Umemura, 2006).

While it is understood that there are effective means of preventing the disease, the number of adults diagnosed with osteoporosis is expected to increase in the next few decades as the "baby boomer" generation ages (NOF 2002; Schneider & Guralnik, 1990). The incidence is predicted to be even greater in postmenopausal women. Women currently and historically have tended to participate in much less athletics and vigorous sports as compared to men. Currently, girls are likely to quit performing vigorous exercise and sports by early adolescence (Kimm, Glynn, Kriska, Fitzgerald, Aaron, Similo, McMahon, & Barton, 2000). Furthermore, adolescents and young adult women rarely report regular vigorous exercise (Kimm, Glynn, Kriska, Barton, Kronsberg, Daniels, Crawford, Sabry, & Liu, 2002). High intensity weight bearing exercise interventions that these young women can easily adopt are greatly needed. Jump training is considered a high impact activity that is easy to perform with minimal time commitment. In fact, simple jump rope, once thought of as only a playground game, has been shown to increase bone parameters more than other weight-bearing exercise in adolescent girls (Arnett & Lutz 2002; Pettersson, Nordstrom, Alfredson, Henriksson-Larsen, & Lorentzon, 2002). Jumping programs have been developed and proven to be effective, however at this time it is necessary to determine the ideal program. In order to determine the most effective program, it is necessary

to develop a more acute method of bone change in the early stages of an intervention.

Rationale

The evaluation of the efficacy of an exercise intervention depends on the precision of the measurement tools. Screening tools need to be capable of identifying changes in bone parameters safely, accurately and efficiently. In addition, these tools need to be used to determine how effective an exercise intervention is early on in the training. Currently, the gold standard for bone diagnosis is dual energy X-ray absorptiometry (DXA). DXA is considered an appropriate measurement tool for detecting changes in BMD in intervention trials of at least four months (Robling, Hinant, Burr, & Turner, 2002; Snow, Williams, LaRiviere, Fuchs, & Robinson, 2001). While DXA is considered safe, the technique does emit ionizing radiation and takes up to 20 minutes to perform a full body scan. Additionally, it is expensive to operate and requires a certified radiology technician to operate the DXA. In 2004, the WHO emphasized that future osteoporosis research should incorporate other technologies such as quantitative ultrasound (QUS) to assess intervention effectiveness (WHO Scientific Group, 2004). Numerous studies have been conducted utilizing QUS in intervention trials (Arnett & Lutz, 2002; Cvijetic, Baric, Bolanca, Juresa, & Ozegovic, 2003; Dib, Arabi, Maalouf, Nabulsi, & Fuleihan, 2005; Daly, Rich, & Klein, 1997; Vignolo, Parodi, Mascagni, Torrisi, De Terlizzi, & Aicardi, 2006; Eliakim, Nemet, & Wolach, 2001). QUS does not emit

ionizing radiation and therefore is considered safe for repeated measures during intervention trials. In addition, the measurement takes only five minutes.

Ultrasound emits a high frequency sound wave, which in turn, can be measured for speed of sound (SOS) and broadband ultrasound attenuation (BUA). These two factors combine to provide Os Calcis Stiffness Index (OCSI) values, which is a measure of bone strength and quality (GE Healthcare, 2004). While ultrasound values are highly correlated (r=0.74-0.88) with osteoporosis diagnostic criteria from DXA (Jaworski, Lebiedowski, Lorene, & Trempe, 1995; Schott, Hans, Sornay-Rendu, Delmas, & Meunier, 1993), QUS measures somewhat different bone characteristics. For example, OCSI provides a measure of trabecular bone architectural and geometric aspects (elasticity, bone stiffness) whereas DXA provides a measure of cortical bone density (Abendschein & Hyatt, 1970; Nurmi-Lawton, Baxter-Jones, Mirwald, Bishop, Taylor, Cooper, & New, 2004). Numerous studies support the claim that OCSI is a good predictor of fracture risk in adults (Arnett & Lutz 2002; Grabe, Cerulli, Stroup, & Kane, 2006; Langton & Langton, 2000; Liu, Xu, Zhu, Han, Zu, & Zhu, 2006; Oral, Tarakci, & Disci, 2006). However, there are no published data concerning the sensitivity of QUS for detecting acute changes in bone following exercise training.

In addition to evaluating bone assessment technologies such as QUS, the WHO also recommended that research efforts utilize

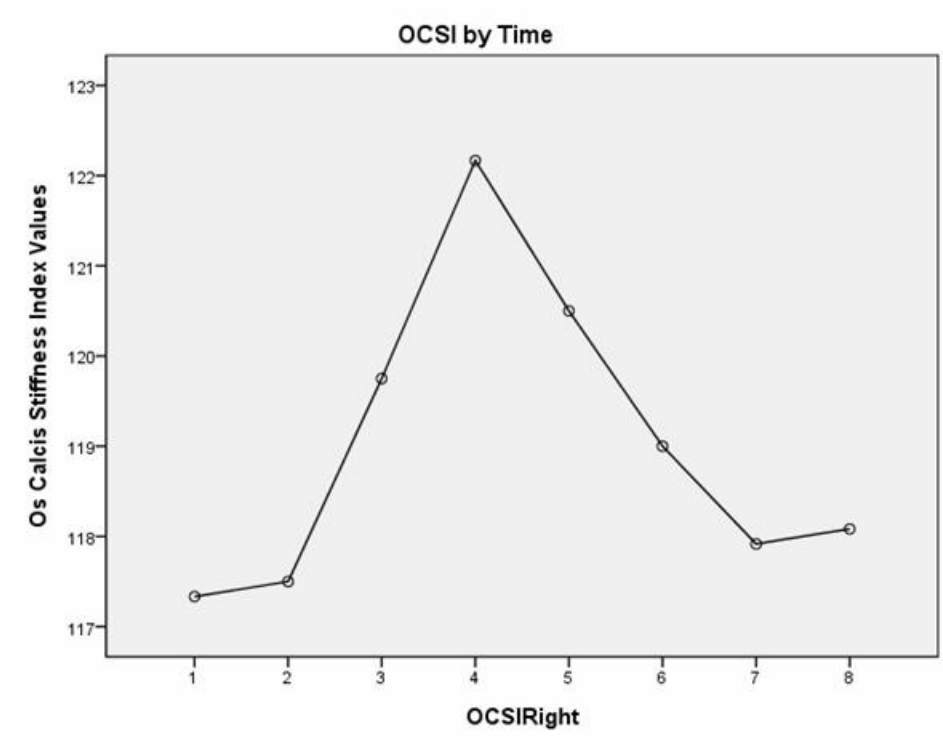
biochemical measures of bone turnover (WHO Scientific Group, 2004). Bone markers of bone status detect change more rapidly than DXA and therefore can be utilized to observe changes in bone status within one month of an intervention (Christenson, 1997). Additionally, biochemical measures of bone remodeling have been determined to be effective at predicting fracture risk and monitoring intervention efficacy (Eastell & Hannon, 2008). There are a variety of assays that can be used to analyze blood markers of bone formation and bone resorption. The reviews that have assessed the utility of biochemical measures of bone turnover have indicated serum osteocalcin as the measure of choice for bone formation (Garnero, 2008). Additionally, significant relationship ($r = 0.90$) have been reported between QUS values and serum osteocalcin (McGehee & Johnson, 2004).

The purpose of this study is to assess the changes in bone following jump training in young women with low bone values as compared to controls. The effects of four months of jump training on bone changes measured by QUS, DXA, and serum osteocalcin are compared to a normally active control group. Comparing serial measures of QUS with serum osteocalcin will provide greater insight as to the sensitivity and thus the usefulness of QUS measures and serum osteocalcin for exercise intervention research. In other words, if OCSI is correlated to serum osteocalcin and is thus an effective predictor of bone formation, then QUS may be used as an early indicator of a program's efficacy. The effects of

exercise training on QUS are unknown. Preliminary data from our laboratory indicated an undulating curve in weekly OCSI values during an 8-week jumping intervention (unpublished, Figure 1). OCSI values increased from week one to four but decreased back to baseline levels by week eight. While QUS changed over time, it is unknown what these responses represent in terms of physiological aspects of the bone. There have been very few previous reports evaluating the relationship between ultrasound and biochemical parameters in a prospective study (Brahm, Strom, Piehl-Aulin, Mallmin, & Ljunghall, 1997). One cross sectional study in endurance trained runners indicated no differences in serum osteocalcin between runners and controls but did find that ultrasound values were higher in runners (Brahm et al., 1997). The cross-sectional nature of this study makes it difficult to determine the relationship between serum osteocalcin and OCSI values. There is no way to know the training status of the runners and whether serum osteocalcin values were indicative of the running at all. To date, there have been no previous studies that have evaluated the effectiveness or sensitivity of QUS or serum osteocalcin as a measure of bone strength during and following an exercise intervention.

Figure 1

OCSI Change Over Time In Jump Training



Primary Aims

1. To determine differences in OCSI (Os Calcis Stiffness Index), serum osteocalcin (a biochemical marker of bone formation), BMC (bone mineral content), and BMD (bone mineral density) in young women with low bone values between two groups: jumping verses controls from baseline to month 4.

2a. To evaluate the individual relationships between 4 bone outcome variables: OCSI, BMC, BMD, and serum osteocalcin at baseline and month four.

2b. To determine if there were between and within group differences over time from baseline to months 1, 2, 3, and 4 in serum osteocalcin and QUS measures.

Null Hypotheses

1. OCSI, serum osteocalcin , BMC, and BMD will not be different between the jumping group and normally active controls from baseline to month 4.

2a. OCSI, serum osteocalcin, BMC, and BMD will not be significantly related to each other at baseline or month 4.

2b. There will not be any significant between group or within group differences in OCSI or serum osteocalcin over time from baseline to months 1, 2, 3, and 4.

Definition of Terms

BMC – Bone mineral content. A measure of bone mass utilizing the DXA reported in grams. Bone mineral content is the accepted means of measuring growth.

BMD – Bone mineral density. A measure of bone density utilizing the DXA reported in g/cm^2. Bone mineral density is the accepted means of measuring an increase in size and mass relative to the individual. T-scores presented from DXA values can be used for absolute change indices.

Body composition – Measurements made to determine the total fat mass and fat-free mass.

BUA – Broadband ultrasound attenuation. A measure of the quantitative ultrasound that determines how quickly sound can reflect upon the bone and return to the same transducer. Measured as transit time by the width crossed, expressed as m/s.

DXA – Dual energy X-ray absorptiometry. The gold standard for bone measurement. This measures bone mineral density and bone mineral content.

FFM – Fat-free mass. Also referred to as lean body mass. The amount of tissue other than fat mass and bone within the body.

FM – Fat mass. The amount of fat tissue within the body.

OCSI – Os Calcis Stiffness Index. A value derived from the Achilles Insight quantitative ultrasound device of the calcaneus. This is value found by using the broadband ultrasound attenuation measurement and speed of sound measurement in an equation to determine the OCSI value.

OST –Serum Osteocalcin – A biochemical measure of bone formation. A protein thought to be secreted by the osteoblasts, responsible for bone building.

QUS – Quantitative ultrasound. A measure of bone strength and quality of bone.

sCTX – Soluble C-terminal peptide. A biochemical measure of bone resorption.

SOS – Speed of Sound. A measure of the quantitative ultrasound that determines how quickly sound can pass through the bone. Related to the elasticity and bone density expressed as g/cm^3.

Delimitations

This study is limited to pre-menopausal women volunteers from 20-40 years of age. Women with t-scores below 1.0 were enrolled. Subjects were required to be healthy without any previous or current conditions known to affect bone metabolism including but not limited to diabetes, thyroid disorders, or being treated with glucocorticoids, anticonvulsants or other drugs known to affect bone metabolism. Subjects also were required to be healthy and capable of performing jumping activities three days per week for up to ten minutes per day. Hormones were not controlled, but subjects were stratified and then randomized by use of birth control pills.

Limitations

This was a convenience sample of volunteers. Genetics were not controlled for in this study. 24-hour dietary recalls were analyzed for calcium intake however direct measures of nutrient intake were not completed. While only normally menstruating women were used, hormonal variation was not controlled. Daily physical activity was monitored through physical activity logs but errors in reporting are possible due to self-report. Because it is unethical to ask subjects to

decrease their physical activity levels, women were asked to continue their normal activity but not change or increase physical activity levels.

Review of Literature

Introduction

Osteoporosis affects approximately 10 million Americans and 75 million people worldwide (Becker, Kilgore, & Morrisey, 2010; WHO Scientific Group, 2003). The incidence rates in the US population results in annual medical costs associated with the disease ranging from 14-20 billion dollars annually (Becker, et al., 2010). Although strong efforts are being made to reduce the risk of osteoporosis and the ensuing fractures, it is estimated that hip fracture incidence rates will increase by 1-3% per year in men and women (Marks, 2010). In fact, NHANES data suggest that US adults in 2005-06 had significantly lower femoral neck bone mineral density (BMD) than were reported from 1988-94 (Looker, Melton, Harris, Borrud, & Shepherd, 2009). The Surgeon General's US Report (2004) warned that by 2020, 50% of the US population over 50 years old would be at risk for a bone fracture (U.S. Department of Health and Human Services, 2004). Concern is therefore growing regarding the development and prevention of this debilitating progressive disease.

There are two interrelated processes involved in the development of osteoporosis: 1) an inadequate development of peak bone mass during youth or 2) a high rate of bone loss through adulthood. During the course of development, bones continue to form and accrue bone mass through growth, remodeling, and modeling. At the onset of puberty, under the influence of hormones, more bone mass is built than in the early

childhood years. This mass and content increases 4-6 times as much from age 11-14 in girls (Javaid & Cooper, 2002). Research has reported that females acquire about 1000 grams of bone mineral mass (40-50% of skeletal mass) and achieve a peak BMD during adolescence (Lloyd, Beck, Lin, Tulchinsky, Eggli, Oreskovic, Cavanagh, & Seeman, 2002). If this work is not done at this time, pharmacological interventions are likely required to try to "freeze" the bone savings accounts so that no further withdrawals can be made from the bone.

In these elderly populations that may require pharmacological interventions, it is currently understood that physically inactive elderly adults are more than twice as likely as their active peers for sustaining a hip fracture. Physical activity provides improvements in muscle strength and therefore decreases the chances of falling and the ensuing lower risk of fracture (Marks, 2010).

Compared to men, women have three times the incidence of osteoporosis (Cawthon, 2011). Forty to sixty years ago girls were not encouraged to participate in athletics or vigorous exercise. Although the emergence of Title IX in the 1970's provided a greater opportunity for women to participate in athletics and vigorous sports, girls still lag far behind boys when it comes to participation in vigorous physical activity. Only later in life do women begin to realize the impact that physical activity can have on their health and they tend to seek exercise opportunities suitable for their physical limitations such as low intensity

activities (swimming, water aerobics, bicycling, etc). A clear u-shaped relationship exists between age and physical activity participation among girls and older women. Girls are likely to quit performing vigorous exercise and sports by early adolescence (Mark & Link, 1999). On the whole, adolescents and young adult women rarely report participation in vigorous exercise (Macera, Ham, Yore, Jones, Ainsworth, Kimsey, & Kohl, 2005). An additional problem is that up to 15% of girls aged 12-19 are not meeting the recommended calcium allowance necessary for peak bone mass attainment (Mark & Link, 1999). Lack of consistent exercise and low calcium intake creates a "perfect storm" for osteoporosis development.

Without intervention and prevention, osteoporosis is predicted to increase over subsequent decades. Prevention of osteoporosis requires a lifetime approach to enhance screening effectiveness and to develop feasible, effective and appropriate evidence-based recommendations for nutrition and weight bearing physical activity.

Figure 2

Projected Trend Of Peak Bone Mass Attainment And Bone Loss

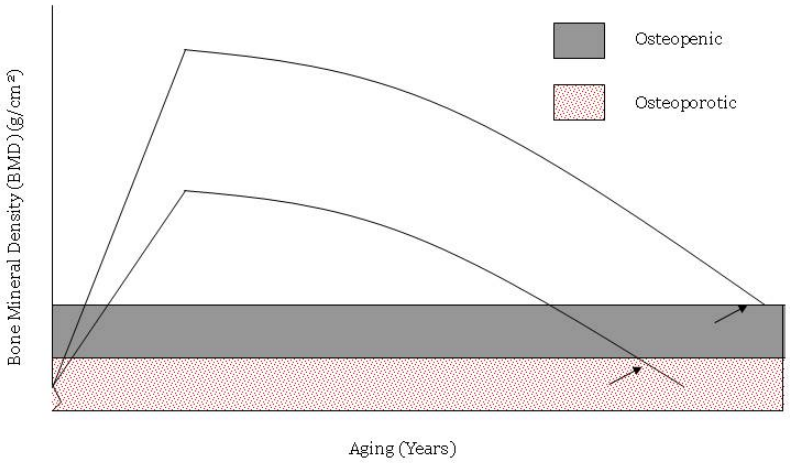


FIGURE 2. This figure depicts the difference between those who attain a higher peak bone mass and the time course of bone loss. Those who attain a higher peak bone mass do not reach the osteopenic level until much later in life than those who do not attain a high peak bone mass. Those who do not attain the high peak bone mass reach an osteoporotic level before the high peak group reaches osteopenic levels (Adapted from WHO, 2004).

Osteoporosis

Osteoporosis is a silent disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a resulting increase in bone fragility (WHO Scientific Group on the Prevention and Management of Osteoporosis, 2003). There are two components of the bone: cortical and trabecular. The density and structure of these bone components will determine its fragility and likelihood of fracture. Cortical bone mass is reported as the density of the bone at a specific site. Trabecular bone is measured to determine its architectural structure and strength by analyzing the number of trabeculae, formation and positioning of the bone

matrix. Over the course of adult life, a depletion of cortical bone mass and weakening of the architecture of the trabeculae leads to bone fragility. Fragility, therefore leads to an increased risk of fracture even in response to small trauma. It is estimated that one in three women over the age of 50 will suffer an osteoporotic fracture in her lifetime (Mark & Link, 1999). Following an osteoporotic fracture, morbidity rates within the first year range from 20-24% (Cooper et al., 1993; Leibson, Tosteson, Gabriel, Ransom, & Melton, 2002). Those who do survive following a fracture also suffer a reduced quality of life due to the disability and possibility for the individual to lose the ability to walk independently (Mark & Link, 1999).

Standard risk categories (t-scores) based on bone mineral density (BMD) values measured by dual energy X-ray absorptiometry (DXA) have been developed as references for levels of normal bone mass, low bone mass (osteopenia), and very low bone mass and bone fragility (osteoporosis). These standard scores have been developed to determine level of fracture risk compared to a healthy young adult population. Each t-score represents a statistical value of one standard deviation on a normal "bell-shaped" distribution curve. Sixty-eight percent of the population therefore fall within one t-score of the median value (normal risk) while those with negative t-scores (< -1.0) are at greater risk for fracture.

According to the World Health Organization (WHO), osteopenia is defined as having BMD t-scores between -1.0 to -2.4, whereas osteoporosis is defined as a t-score below -2.5 (WHO, 2004). A diagnosis of osteopenia

is therefore considered to be a clinical precursor to osteoporosis. If not screened, diagnosed, and treated for osteopenia, osteoporosis likely will be the result. Recently, the osteoporosis screening recommendations (National Osteoporosis Foundation, 2010) were updated. These new recommendations stipulate that BMD assessment is recommended for women over age 65 and men over age 70 unless there are other clinical reasons to assess bone status. While assessing BMD at these ages may clearly improve the diagnosis of osteoporosis, the recommendations are not likely to be helpful for early detection of osteoporosis. Interestingly, although the statement also recommends prevention of the disease, no proactive approach was suggested to assess bone status during youth and adolescence when true prevention may actually be possible (Foley, Quinn, Dwyer, Venn, & Jones, 2008).

Peak Bone Mass

Measuring peak bone mass (PBM) is an additional means of predicting risk of fracture later in life. Regardless of age, elevating a person's peak bone mass one standard deviation above their current levels would reduce the risk of fracture by 50% (Kriemler et al., 2008). Thus facilitation of the highest peak bone mass possible is considered a preventive measure.

Age of PBM attainment is dependent upon skeletal site and the measurement tool used for assessment. In a longitudinal study of a 16-40 year old Canadian population, lumbar spine PBM occurred at 33-40 years

in women and 19-33 in men whereas hip PBM occurred much earlier at 16-19 years in women and 19-21 in men. Although attainment of peak bone mass is known to be highly heritable (Ralston & Uitterlinden, 2010), Frost (2000) reported that muscle forces acting on the bone have a greater effect on developing peak bone mass than genetics or non-mechanical factors such as hormones and calcium intake (Frost, 2000). Therefore, exercise has been highly studied for its role in increasing peak bone mass attainment.

Jumping is a common activity utilized in interventions to increase bone mass in children (Arnett & Lutz 2002, Gunter et al., 2008a; Gunter, Baxter-Jones, Mirwald, Almstedt, Fuller, Durski, & Snow, 2008b; Johannsen et al., 2003; McKay et al., 2005a; Weeks et al, 2008). The relationship of lean mass and isokinetic peak torque also has been positively correlated with bone mass, geometry and strength in prepubertal girls (Daly, Stenevi-Lundgren, Linden, Karlsson, 2008; Heinonen, McKay, Whittall, Forster, & Khan, 2001). The maximal isometric voluntary contraction of the arms and legs was highly correlated ($r^2 = .54$) with BMC (Wang, Alen, Nicholson, Suominen, Koistinen, Kroger, & Cheng, 2007). This relationship between strength and BMC was even more apparent throughout the pubertal growth spurt (Wang et al., 2007).

However, high levels of physical activity are contraindicated in women who meet the characteristics of the female athlete triad

(amenorrhea, low calorie intake/disordered eating, thus leading to a low bone mass) (Iwamoto, Sato, Takeda, & Matsumoto, 2009). In a study comparing female high school athletes who were eumenorrheic (normally menstruating) to those who were amenorrheic (not menstruating) or oligomenorrheic (irregularly menstruating), those with a normal menstrual function had significantly greater total hip and trochanter BMD than their oligo/amenorrheic counterparts (Nichols, Rauh, Barrack, & Barkai, 2007). Therefore, care needs to be taken to ensure that females have normal menstrual function so they do not diminish their potential to attain a high peak bone mass with exercise.

Bone Loss

Bone loss rates are less genetically determined than attainment of peak bone mass (Ralston & Uitterlinden, 2010). Thus the heritable risk for developing osteoporosis may be more dependent on the peak values obtained than on the genetically determined rate of bone loss. Women who do not reach above normal t-scores early in life will lose enough bone to reach a clinically at-risk level by menopause (Figure 2). Typically women lose bone at a rate of approximately 1% per year at pre-menopause, and 4% per year after menopause (WHO, 2004). Since the average life expectancy for a woman in the US is 80 years old, then with this rate of loss, women would be susceptible to more fragile bones, significantly increasing her risk of fracture and disability.

Snow and Marcus (1991) reported that bone loss, from its peak to age 80, is comparable to the reported 35-45% decline in muscle strength observed over this same period of time (Snow & Marcus, 1991). In two large cohort studies, lean body mass was the strongest predictor of BMD in the cohort of children (Falkner, Bailey, Drinkwater, Wilkinson, Houston, & McKay, 1993), in young pre-menopausal, peri-menopausal and early menopausal women (Ilich-Ernst, Brownbill, Ludemann, & Fu, 2002). However, lean body mass alone is probably not the best predictor of BMD. Beck and Snow (2003) showed that elite swimmers with plenty of muscle mass, who spend over 20 hours per week in a buoyant environment, actually unload their skeletons (Beck & Snow, 2003).

Factors Related To Peak Bone Mass And Loss.

Bone regulation is dependent on modifiable (behavioral) and non-modifiable (genetic) factors. Heredity, endocrine, mechanical and nutritional factors affect the ability to attain a high peak bone mass and maintain that mass throughout life. These factors are intertwined and interdependent for the development of peak bone mass. The modifiable factors such as dietary intake of calcium, Vitamin D, and phosphates, as well as physical activity levels, body weight and smoking behaviors, account for a significant portion of variability in peak bone attainment (Heaney, Abrams, Dawson-Hughes, Looker, Marcus, Matkovic et al., 2000).

Heredity.

As previously noted, the contribution of heredity on bone loss differs from peak bone mass attainment. Ralston (2010) emphasized that there is a greater heritable influence on peak bone mass attainment than the subsequent loss of bone mass over the lifespan (Ralston, 2010). Genetics accounts for a sizable amount (50-80%) of peak bone mass attainment (Root, 2002; Bonjour, Chevalley, Ferrari, & Rizzoli, 2009; Ferrari, 2008). Research is ongoing to identify the genes responsible for influencing both peak bone mass attainment and bone loss. The receptors that influence bone metabolism have been identified and therefore have been targeted with osteoporosis drugs such as bisphosphonates. These different receptors affect different aspects of bone tissue and bone metabolism. The heritability of fracture ranges from 25-48% whereas the heritability of bone turnover ranges from 40-70% and hip geometry from 70-85% (Ferrari, 2008).

In regard to heritability of osteoporotic fractures, women who had a mother who suffered a hip fracture were twice as likely to suffer the same type of fracture as someone without a family history (Kanis, Barlet, Cooper, Delmas, Reginster, Borgstrom, Rizzoli, & ESCEO, 2008). However, in another study, adult mothers and daughters shared no relationship in their levels of BMD. In fact, the mother's total BMD was positively associated with supplemented calcium intake, body weight, estrogen replacement therapy use, and past oral contraceptive use and

negatively with age and height. The daughter's (mean age 41) levels of lifetime weight bearing physical activity was the strongest predictor of total and peripheral BMD and their total lean mass was a predictor of their axial BMD (Ulrich, Georgiou, Snow-Harter, & Gillis, 1996). Similarly, a recent study of Japanese adolescent daughters, mothers and grandmothers examined the heritability of bone status. While BMD was significantly correlated between the mothers (mean age, 46.4 years) and daughters (mean age, 14.6 years), no correlation existed between the daughters and grandmothers (mean age, 71.9 years) or mothers and grandmothers (Ohta, Kuroda, Onoe, Nakano, Yoshikata, Ishitani, Hashimoto, & Kume, 2010). Therefore, although relationships appear to exist between mother daughter pairs prior to menopause, many other factors appear to affect this relationship after menopause. Clearly, the influence of heredity can influence specific processes but the interaction of lifestyle with aging plays a significant role in peak bone mass and bone loss.

Endocrine factors.

Hormones, both endogenous and exogenous, also play a critical role in peak bone mass attainment. The reproductive hormones that are typically attributed to changes in the skeleton may not have independent effects on the skeleton. It appears that a single change in a given hormone in the hypothalamic-pituitary-gonadal (HPG) axis acts to affect and disrupt the entire feedback loop (Nicks, Fowler, & Gaddy, 2010). This

feedback loop includes estrogen, progesterone, androgens, follicle-stimulating hormone, pituitary luteinizing hormone, prolactin, and oxytocin.

Estrogen is the factor directly related to the peak development of bone mass at puberty and the loss of bone mass at menopause (Nicks et al., 2010). During growth, estrogen is an essential component of longitudinal bone growth, accelerating bone growth at the start of puberty and is also responsible for the closing of growth plates (Bonjour et al., 2009). After menopause, fat tissue is the primary source of endogenous estrogen. However, its role in pre-menopausal women is not completely understood.

In a study examining predictors of hip and spine BMD, lean body mass was the strongest independent predictor of both hip and spine BMD whereas luteal phase serum concentrations of estradiol, progesterone, and testosterone were not predictors of BMD (Lu, Nayeem, Anderson, Grady, & Nagamani, 2009). The authors suggested that the endogenous estrogen in ovulating women may be sufficient to maintain bone mass.

Lifetime exposure to estrogen is another consideration for a predictor of BMD. It is considered to be reasonable that the earlier one reaches menarche, the higher their BMD, but there is no direct evidence to verify this explanation (Bonjour et al., 2009). In a cross sectional study, menarche at age 15 or later was found to be an independent predictor of low bone mass when measured using DXA and QUS for measurement

(Hawker, Jamal, Ridout, & Chase, 2002). Therefore, while estrogen may appear to have a direct relationship with bone mass, there are likely other factors that play a role in peak bone mass attainment and the loss of bone.

Mechanical factors.

Various exercise modalities have proven to be effective at increasing PBM or preventing bone loss. A meta-analysis of high-intensity resistance training displayed a significant increase in total BMD and increases in femoral neck BMD (Martyn-St James & Carroll, 2006). The effects of weight-bearing activity on bone are reasonably well-known. However, it is unclear what types and at what age this training is most effective. Multiple training modalities have been used to treat and prevent osteoporosis. Jumping has been shown to be effective in young girls (Arnett & Lutz 2002, Gunter et al., 2008a; Gunter et al., 2008b; Johannsen et al., 2003; McKay et al., 2005a; Weeks et al., 2008), young adults (Kato et al., 2006) and postmenopausal women (Snow, Shaw, Winters, & Witzke, 2000). These types of interventions vary by the type of jumping performed and population utilized. Plyometric training, jumping in place, and jump roping have all been shown to be effective in improving bone parameters (Arnett & Lutz, 2002, Gunter et al., 2008a; Gunter et al., 2008b; Johannsen et al., 2003; Kato et al., 2006; McKay et al., 2005a; Snow et al., 2000, Weeks et al., 2008).

In an intervention involving 100 box jumps three times per week for seven months, prepubescent children who jumped had significantly

greater BMD than controls at the spine and this greater improvement in BMD approached significance at the femoral neck (Fuchs, Bauer, & Snow, 2001). The same effect was seen in all BMC sites after a similar duration and frequency intervention of 100 jumps performed in the PE classroom (Gunter et al., 2008a). This effect has also been observed in early pubertal children with as little as 10 jumps, three times per day, five days per week (McKay et al., 2005a). Circuit training performed within the school day for two years also significantly increased hip and spine BMC over controls (MacKelvie, Khan, Petit, Janssen, & McKay, 2003). Resistance training alone has also been shown to increase femoral neck BMD over controls in an adolescent population (Nichols, Sanborn, & Love, 2001). In the long-term a study analyzed postmenopausal women who jumped with weighted vests three days per week, for 32 weeks per year over five years. This intervention was effective at significantly preventing bone loss (Snow et al., 2000).

Some questions have been raised as to whether this training effect can be maintained if activity is terminated. Three groups have reported that an increase in bone mass can be maintained after detraining (Beck & Snow, 2003, Fuchs & Snow, 2002; Gunter et al., 2008a). In contrast, questions have been raised regarding the ability to increase BMD or maintain this BMD with low to moderate intensity levels of PA. Hagberg and colleagues (2001) showed that prolonged low- to moderate intensity physical activity was independently associated with a higher BMD

(Hagberg, Zmuda, McCole, Rodgers, Ferrell, Wilund, & Moore, 2001). The effects of walking also continues to be researched to determine if this mode of activity can be used for at least the maintenance of bone in the older age groups at risk for bone loss. In studies by Kitagawa (2003) and Yamazaki (2004), it appears that walking can prevent bone loss associated with aging (Kitagawa, Omasu, & Nakahara, 2003, Yamazaki, Ichimura, Iwamoto, Takeda, & Toyama, 2004).

Nutritional factors.

Calcium and vitamin D are the two primary factors related to bone metabolism. Calcium intake is encouraged in high dietary doses (>1000 mg/day). Some research has shown dose response relationships with calcium intake and BMD in children and halted bone loss in older adults with higher calcium intake (Heaney, 2000). However, in a model created to determine the amount of variance in bone mass explained by modifiable determinants, calcium intake only explained 1-4.7% of the variance (Lloyd et al., 2002). If however, dietary calcium is not adequate, bone resorption is the result, causing bone calcium to be released from the bone into the blood (Heaney, 2000). In a calcium supplementation study in prepubertal children, only those who were low in calcium intake at baseline displayed significant improvements in BMD with supplementation (Bonjour, Carrie, Ferrari, Clavien, Slosman, Theintz, & Rizzoli, 1997). This same effect of calcium supplementation on those with low baseline calcium intake has also been shown in late adolescence (Rozen, Rennert, Dodiuk-Gad,

Rennert, Ish-Shalom, Diab, Raz, & Ish-Shalom, 2003). However, the effect of supplementation was less robust in late adolescence than in the pre-pubertal study (Vatanparast & Whiting, 2006). In late postmenopausal women, bone loss can be reduced with 500 mg per day of calcium carbonate supplementation (Dawson-Hughes, Dallal, Krall, Sadowski, Sahyoun, & Tannenbaum, 1990). While calcium alone cannot support bone health (Morgan, 2009), clearly there is need for an adequate calcium intake. However, any dose response relationship of calcium intake above the recommended levels is co-dependent on other dietary and hormonal factors.

Vitamin D levels are considered a primary factor in attaining peak bone mass (Lanham-New, 2008). However, 75% of the US Caucasian population is considered to be vitamin D insufficient as are 90% of the pigmented population (African-Americans, Hispanics, and Asians) (Adams & Hewison, 2010). In 9-15 year old girls, those in the highest tertile of vitamin D intake had 27% higher lumbar spine BMD than those in the lowest vitamin D tertile (Cheng, Tylavsky, Kroger, Karkkainen, Lyytikainen, Koistinen, Mahonen, Alen, Halleen, Vaananen, & Lamberg-Allardt, 2003). In a five-year randomized trial, vitamin D plus calcium pill supplementation was shown to have long-term beneficial effects on bone density (Zhu, Devine, Dick, Wilson, & Prince, 2008). Furthermore, in a meta analysis of 29 randomized trials, supplementation with calcium and

vitamin D3 reduced the risk of bone fracture by 24% and significantly reduced bone mass loss (Stransky & Rysava, 2009).

Other nutrients also play an important role in bone metabolism in relation to bone building and the prevention of bone loss. Adequate intake of protein, and vitamin K need to reach recommended levels to prevent impaired bone synthesis (Root, 2002). Although vitamin K plays a role in osteocalcin formation, supplementation appears to offer no additional benefits beyond calcium and vitamin D supplementation (Morgan, 2009). Low circulating vitamin K however has been reported to be associated with low BMD and higher risk of fractures (Ilich & Kerstetter, 2000). Protein supplementation was shown to reduce bone loss in two studies of older people (Darling, Millward, Torgerson, Hewitt, Lanham-New, 2009). In younger populations, protein supplementation with whole milk consumption (milk basic protein, (MBP)), significant increases in total BMD were observed. Overall, it appears that an adequate diet is needed and will be effective in preventing bone loss and maximizing peak bone mass.

Conclusion to Peak Bone Mass and Loss.

The evidence is clear that the critical time to aid in the prevention of osteoporosis is during adolescence, ideally from the pubertal growth spurt to the third decade of life. Although heredity accounts for a large portion of peak bone mass and the rate of bone loss, there is still large variability that can be affected by lifestyle factors. The primary goal for researchers

and health care professionals is to maximize bone mass during puberty and adolescence, prevent bone loss, and postpone the occurrence and time spent living with fragile bones. Researchers need to develop and evaluate training models for the prevention of this disease and determine effective interventions and programs that can be utilized successfully by the healthcare community.

Bone Physiology

The function of bone is to serve as the structure and protection for the human body. About 40 years ago, researchers began to realize that bone is not a dormant tissue and the process of bone building is a complex process (Galli, Passeri, & Macaluso, 2010). It is important to note that although the basic functional cells of bone (i.e. osteocyte and osteoblast) were first discovered in the 19th century (Iqbal, Sun, Zaidi, 2010), the process by which these cells communicate and regulate bone turnover is not fully understood and is currently the focus of a substantial amount of research.

Three Main Factors.

The osteocyte or bone cell, was originally observed as a metabolically inactive cell and was referred to as simply a "placeholder" within the bone tissue (Brandi, 2009). However, now it is clearly established that the osteocyte is a critical component of the complex process of bone maintenance and osteoblastic and osteoclastic communication. Osteoblasts and osteoclasts are generally accepted as the

bone building, and bone break-down elements of bone turnover, respectively. Osteoblasts are cells that function to synthesize and mineralize the osteoid (newly mineralized bone cell), as well as produce factors that regulate the osteoclast activity. Osteoclasts are bone resorbing cells (Brandi, 2009). They regulate the activity of the osteoblastic cells and a host of other processes such as the activity of hematopoietic stem cells. Osteoclasts secrete various cytokines and can act as immune cells in inflammatory bone diseases (Boyce, Yao, & Xing, 2009).

Osteocyte.

The osteocyte is a terminally differentiated osteoblast placed in the bone matrix. Osteocytes themselves are connected to one another and osteoblastic cells on the bone surface by dendritic processes that reside in canaliculi (tiny canals), which contain the bone extracellular fluid. The dendritic processes are in contact with bone marrow, allowing them the ability to recruit osteoclast precursors to stimulate bone resorption and regulate mesenchymal stem cell differentiation. The purpose of the osteocyte is to act as a mechanosensor in the bone, sensing physical strain and initiating the appropriate modeling or remodeling responses (Brandi, 2009), thereby likely determining which bone surface the osteoclast will resorb (Matsuo & Irie, 2008).

Over 90% of all bone cells are composed of osteocytes in the adult skeleton. Osteocytes have the unique ability to sense the intensity of mechanical strain and respond by sending signals through the canaliculi

or canals in the bone to the osteoblasts and osteoclasts to regulate bone modeling or remodeling (i.e., bone turnover) (Bonewald & Johnson, 2008). This process is especially necessary for maintaining bone mass in response to a normal load. In fact, in the absence of a skeletal load, the osteocyte is responsible for signaling bone resorption from the osteoclasts (Bonewald & Johnson, 2008). Thus, constant mechanical strain is necessary for continued bone turnover, development, and maintenance.

Lineage.

The osteoclast and osteoblast each have their own specific lineage. Each cell line can communicate and be regulated by: cell-to-cell contact, diffusible paracrine factors and cell-bone matrix interaction. Osteoclast lineage is derived from blood forming (hematopoietic) stem cells and share precursors with macrophages. Osteoblast lineage includes: stromal cells, bone lining cells, osteoprogenitors, preosteoblasts, osteoblasts, and osteocytes. The osteocytes are derived from mesenchymal stem cells which then differentiate into fibroblasts, chondrocytes, myoblasts, and adipocytes (Matsuo & Irie, 2008).

Communication.

The process of communication between osteoblasts, osteoclasts, and osteocytes can be direct or indirect and results from the unique properties of these cells. The osteoblast and osteoclast can communicate with each other in at least four ways. 1. The osteoclast and osteoblast can make direct contact with one another through membrane bound ligands

(the molecule that has bound to the receptor), allowing the attached molecule and receptor to interact and initiate intracellular signaling. 2. The two can form gap junctions (channels) allowing passage of small water-soluble molecules between the two different cell types. 3. During bone resorption, osteoclastic activity may liberate growth factors and other molecules in the bone matrix that were previously deposited by osteoblasts. 4. By responding to diffusible paracrine factors, such as growth factors, cytokines, chemokines and other small molecules secreted by either cell type and acting on each other via diffusion (Matsuo & Irie, 2008).

Modeling and Remodeling of Bone.

There are two phases of bone change that occur within the body: bone modeling and remodeling. Modeling occurs as a normal function of growth but also in the adult in direct response to a mechanical load and stimulus. Bone remodeling takes place in response to various stimuli such as the generation of bone microcracks, loss of mechanical loading, low blood calcium, or alterations in hormones and cytokines (Matsuo & Irie, 2008).

Bone modeling.

Modeling is independent of and not directly paired with bone resorption from osteoclastic activity. Manipulation of bone size and shape is typically seen in youth but can occur in adults in response to a significant overload. An example of modeling in adults is observed in

response to bisphosphonate treatment because osteoclastic activity is inhibited. Modeling occurs much less than the remodeling process; however the occurrence of modeling can be increased in some pathological states. The majority of research is focused on the bone remodeling process (Brandi, 2009).

Bone remodeling.

The remodeling process is a "surface-based phenomenon" that involves the removal of bone by osteoclasts followed by the deposition of a new bone by osteoblasts in the newly formed cavity. Approximately 10% of the bone surfaces in the adult skeleton are undergoing remodeling at a given time. This process is said to take about six months, of which most of the time is spent in the formation process (Brandi, 2009).

Figure 3

Stages Of Bone Remodeling

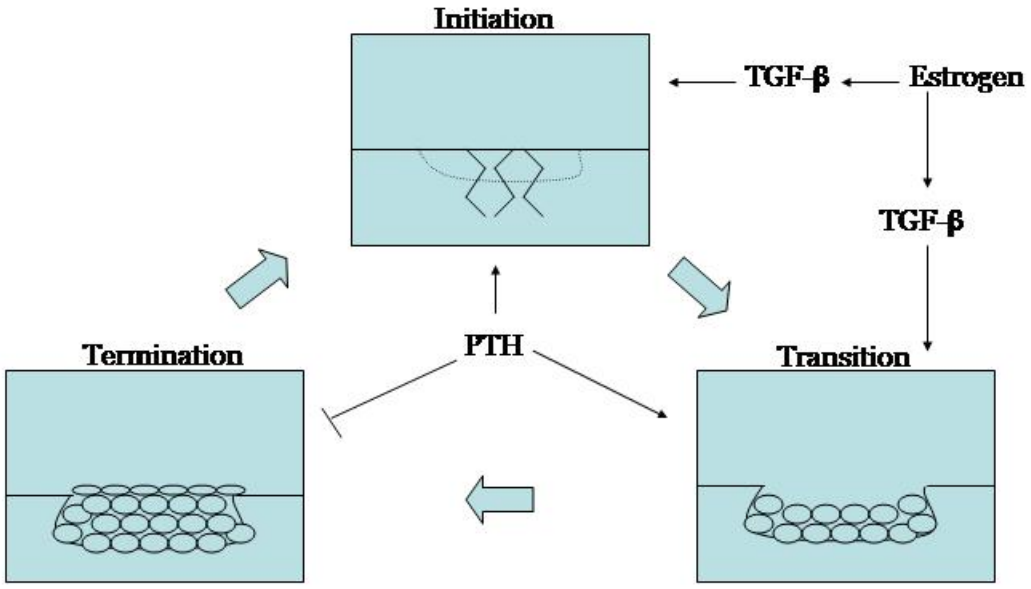


Figure 3. Initiation phase begins due to microcracks on the bone surface. Bone resorption begins due to osteoblasts binding to osteoclast precursors. In the Transition phase, the new surface is cleaned and bone formation begins to fill the new surface due to coupling factors. The Termination phase ensures that the new space is filled and covers the surface to form a new layer of cells over the newly formed bone. PTH (parathyroid hormone) acts on each stage of the process, increasing bone resorption and formation and blunting further formation or resorption in the termination phase. Estrogen acts on the Initiation and Transition phase by increasing the secretion of TGF-β.

(Matsuo & Irie, 2008)

The process of bone remodeling consists of a cyclical series of events represented in Figure 3. These events are named differently throughout the literature; however for the purposes of describing specifically osteoblast, osteoclast and osteocyte intrinsic communication a three step process will be described. The cross-talk between osteoblasts and osteoclasts is represented in this process: (A) Initiation, (B) Transition, and (C) Termination. The directions of communication are opposite one another between the initiation and transition phases. During

initiation, osteoblasts are directed to recruit and thereby bind to osteoclastic precursors (thought to be done by stromal cell-derived factor (SDF-1), whereas during the transition phase, osteoclasts are directed to osteoblastic precursors (Matsuo & Irie, 2008). The osteoclast and osteoblast are all components of the basic multicellular unit (BMU) but this unit is not complete without the osteocyte and immune cells (Matsuo & Irie, 2008; Seeman, 2009). It is important to also note that RANK and RANKL are two important factors that signal for the osteoclast precursors and osteoblast precursors respectively and are essential for the development of osteoclasts. Osteoclast precursors are attracted to the sites of bone microcracks where bone resorption will occur. Here, they fuse with one another to form the multinucleated cells that resorb calcified matrixes. They also regulate the differentiation of osteoblast precursors and the movement of hematopoietic stem cells from the bone marrow to the bloodstream (Boyce et al., 2009).

Figure 4

Initiation Phase

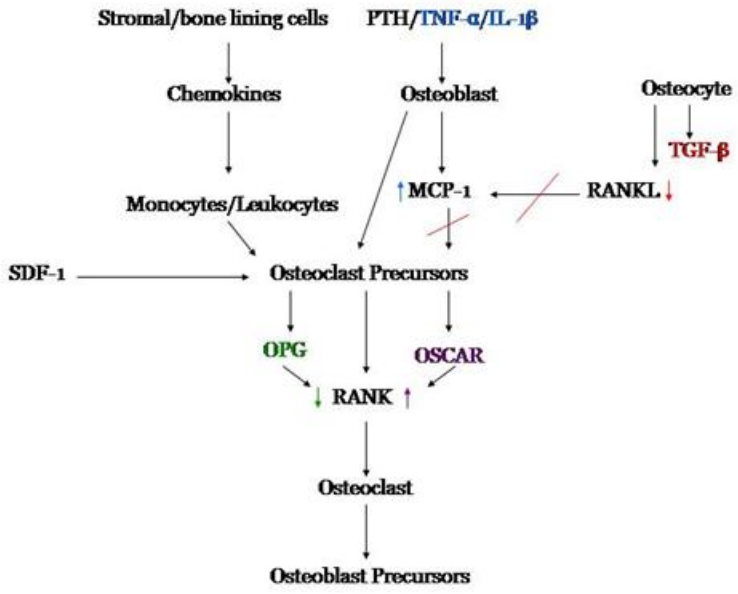


Figure 4. Multiple variables recruit osteoclast precursors. Stromal/bone lining cells secrete chemokines which recruit the monocytes and leukocytes to recruit the osteoclast precursors. PTH (parathyroid hormone), TNF-α, and IL-1β recruit osteoblasts, which secrete MCP-1 (which is thought to be increased by IL-1β, to recruit osteoclast precursors. Osteocytes also recruit RANKL to secrete MCP-1 to recruit osteoclast precursors. However, in response to stress, the osteocyte will release TGF-β, thereby reducing the release of RANKL and reducing osteoclast precursor recruitment. Osteoclast precursors secrete OPG and OSCAR to secrete RANK. OPG decreases RANK secretion and OSCAR increases RANK secretion. RANK then creates osteoclasts that recruit and bind to the osteoblast precursors.

Initiation phase.

The Initiation phase involves the recruitment of osteoclast precursors, differentiation, activation of osteoclasts, and bone resorption. This process takes approximately three weeks. In this phase, the cells attach to a bone surface that was previously quiet, and it changes to a remodeling surface. Here, the initiation of osteoclast creation depends upon the communication between the osteoclast precursor cells and the cells of the osteoblast lineage. Chemokines, (chemotactic cytokines) start the recruitment of monocytes and other leukocytes and are likely secreted

by stromal or bone lining cells to recruit osteoclast precursors (Matsuo & Irie, 2008).

MCP-1 (monocyte chemotactic protein-1), created by osteoblasts is also thought to recruit osteoclast precursors. MCP-1 is secreted by the osteoblast in the absence of inflammation or in response to parathyroid hormone (PTH). MCP-1 is also thought to be produced in response to TNF-α and Interleukin-1β (IL-1β). Osteoblast induced recruitment utilizing MCP-1 is thought to be enhanced through RANKL (also known as TRANCE) secretion by the osteocyte. If the osteocyte needs to respond to a physiological mechanical stress, it is thought that it releases TGF-β, thereby reducing the secretion of RANKL and preventing osteoclast creation. RANK is also secreted by the osteoclast precursor cells to aid in the creation of osteoclasts. The osteoclast precursor cells are attracted to sites of bone resorption, form multinucleated cells that resorb the calcium matrix, and regulate differentiation to hematopoietic stem cell from the bone marrow to the bloodstream. Osteoclast precursors also excrete immunoreceptors, such as OSCAR, that work with RANK to send osteoclast signals. A third factor, OPG (Osteoprotegerin), inhibits RANK signaling by acting as a decoy to RANKL, thereby interrupting osteoclast-osteoblast communication (Matsuo & Irie, 2008).

Transition phase.

Multiple forms of communication occur between bone cells in order for the bone to be resorbed, and for formation to occur. Figure 5 displays the pathways that can lead to bone formation.

Figure 5

Pathways To Bone Formation

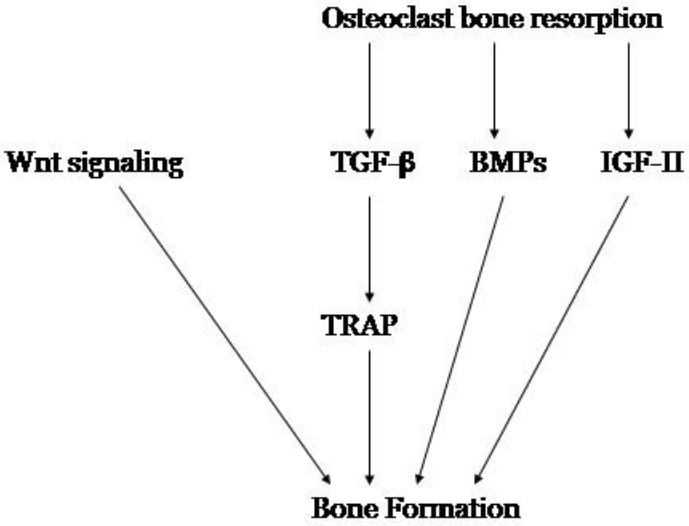


Figure 5. When osteoclast bone resorption takes place, TGF-β, BMPs (bone morphogenic proteins), and IGF-II are released which trigger bone formation. It is understood that TRAP (type V tartrate-resistant acid) may also stimulate bone formation, but that TGF-β may bind to TRAP to stimulate bone formation. Wnt signaling is also known to start the process of bone formation.

During the transition phase, coupling of the osteoclast and osteoblast is critical. The bone-resorbing osteoclast stimulates differentiation of the osteoblast precursors, thereby activating bone formation in bone resorption lacunae, spaces occupied by osteocytes that

have migrated into and become trapped and surrounded by the bone matrix.

Figure 6

Osteoclast/Osteoblast Communication

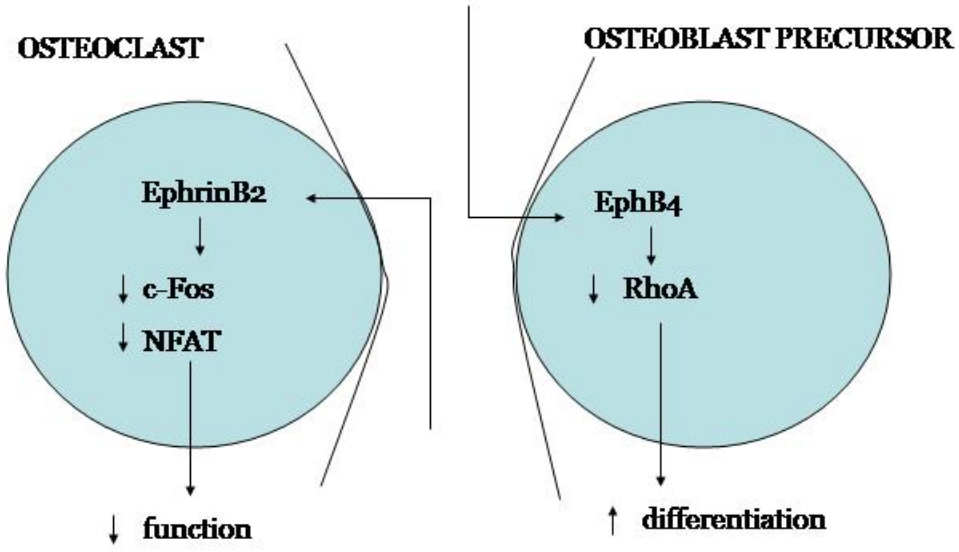


Figure 6. Adapted from Matsuo & Irie, 2008. EphrinB2 enters the osteoclast via reverse signaling, reducing the production of c-Fos and NFAT, thereby reducing the osteoclast function. EphB4 enters the osteoblast precursor via forward signaling, reducing Rhoa activity and increasing osteoblast precursor differentiation. This starts the process of formation.

This process is commonly referred to in the literature as a separate phase termed "reversal." During this segment of the transition phase, EPH tyrosine kinase receptors and ligands work to suppress osteoclasts and enhance osteoblast precursors. This reverse signaling is initiated by the ephrinB2 ligand that reduces c-Fos and NFAT activities thereby reducing the function of the osteoclast. The forward signaling of the Eph4 receptor reduces RhoA activity and thereby increases osteoblast precursor

differentiation. This process is thought to be the point where the balance between EphrinB2 and Eph4 interactions assist in the transition from the Initiation to the Transition phase (Matsuo & Irie, 2008).

While bone formation is starting, osteoclast bone resorption stops and osteoclasts undergo apoptosis, programmed cell death, caused by a high amount of extracellular calcium being released from bone during resorption. Coupling, the point when bone resorption and formation are balanced, occurs in the BMU. This coupling occurs once free, secreted, or membrane-bound molecules produced by osteoclasts act on the osteoblast precursors to stimulate bone formation. In addition, the number of osteoclasts, not the bone resorption itself is critical to stimulate bone formation and can occur without cell to cell contact. The bone resorption however may free growth factors such as TGF-β, bone morphogenetic proteins and IGF-II from the bone matrix thereby activating osteoblastic bone formation. The BRC, a compartment in the bone marrow, consists of a canopy of lining cells and the BMU underneath on the bone surface. This structure therefore ensures osteoclast and osteoblast communication. Here, cell-to-cell contact between osteoclast and lining cells of the osteoblast lineage on top of the osteoclasts in the BRC may occur along with side-by-side contacts on the bone surface. Gap junction communication occurs between osteoblasts mediated by connexin that stimulates osteoblast differentiation and bone formation without physical contact with the adjacent cells. These hemichannels (the halves of gap

junction channels that do not require physical contact), provide a mechanism for ATP (adenosine triphosphate) and NAD^{+} (nicotinamide adenine dinucleotide) release, which results in elevated Ca^{+2} (calcium) levels (Bonewald & Johnson, 2008). The resulting effect of elevated calcium levels are increased osteoclast apoptosis. However, connexin acts on osteoclasts to aid in osteoclast survival and formation. This gap junction communication may likely occur only when the osteoblast and osteoclast cells are next to one another (Matsuo & Irie, 2008). This careful balance of osteoblast and osteoclast communication leads to the termination phase.

Termination phase.

During the termination phase, osteocytes produce sclerostin (Sost) which suppresses osteoblastic bone formation. At this point, the osteoblast becomes quiet, likely due to the help of Sost that is secreted through the osteocyte canaliculi. In this phase, osteoclast differentiation is suppressed, probably through the OPG (osteoprotegerin) produced by osteoblasts. The canonical Wnt signaling through β-catenin activates this OPG gene promoter in the osteoblasts, thereby inhibiting osteoclast development. There is possibly an interaction between osteoclasts and osteoblasts that induces notch signaling in osteoblasts, leading to an increased OPG production and reducing osteoclast development. Therefore, Wnt signaling, EBF2 activity, and notch signaling work together to slow osteoclast creation by inhibiting RANK signaling through

the secretion of OPG by mature osteoblasts. OPG function then works to aid in the initiation phase, (where it was first discussed due to its function with RANKL for acting to increase osteoclast formation) (Matsuo & Irie, 2008). It has been suggested by Bonewald and Johnson (2008) that the regulation of the OPG/RANKL ratio is one potential means by which formation and resorption can be maintained in equilibrium.

As OPG is secreted, we can expect for the initiation phase to start in another area of the bone surface where this 3-phase process will continue to repeat and remodel the bone.

Factors Affecting Remodeling.

Mechanical strain.

This process continues assuming that an average amount of mechanical strain is placed upon the skeleton. Mechanical strain can be sensed by bone lining cells, osteoblasts, and osteocytes, and translate this into biochemical responses. Osteocytes also appear to be the most capable of sensing and differentiating with various intensities of strain (Bonewald & Johnson, 2008). These researchers have also proposed that muscle contractions are responsible for basal transport of nutrients to the osteocytes and that stresses due to exercise can be superimposed.

Extrinsic factors.

Extrinsic factors such as parathyroid hormone, vitamin D, estrogen, growth and thyroid hormones, and glucocorticoids all act to alter the bone remodeling process. These factors function to increase bone resorption,

prevent further bone resorption, increase bone formation, and prevent further bone formation. In some cases, high exposure to specific factors can have a contradictory affect on formation or resorption. The common extrinsic factors that affect bone will be discussed briefly. Parathyroid hormone functions to regulate serum calcium concentrations. It is considered a potent stimulator of bone resorption. As a person ages, their plasma PTH will increase and may therefore increase bone turnover with an end result in a loss of bone mass, especially cortical bone mass.

Vitamin D ($1,25(OH)_2$) will also increase bone turnover and could decrease bone formation in large doses. It is beyond the scope of this paper to discuss at length the effect of vitamin D on bone turnover however vitamin D has its greatest effect on intestinal calcium and phosphate absorption. Vitamin D levels are considered a key signal in the differentiation of bone osteoblasts and osteoclasts and activation of bone turnover (Raisz, 1999).

Estrogen levels tend to increase bone turnover with an overall net gain of bone mass because it is hypothesized that the resorption is counter balanced with formation. Estrogens act on at least five intrinsic factors to indirectly positively or negatively affect bone resorption and formation (Raisz, 1999). For example, during hormonal therapy, estrogen supplementation is thought to prevent osteocyte apoptosis via its action on TGF-β (Henriksen, Neutzsky-Wulff, Bonewald, Karsdal, 2009). However, a deficiency of estrogen is known to result in osteocyte apoptosis which

results in damage and bone fragility (Seeman, 2009). The effect of androgens and growth hormone are not completely known. They both tend to increase bone resorption and bone formation. Thryoid hormones also increase both bone resorption and formation. Glucocorticoids increase bone resorption and decrease bone formation, also resulting in osteocyte apoptosis, which again, produces bone damage and can result in fragility (Raisz, 1999; Seeman, 2009). Patients taking moderate to high doses of these drugs require careful monitoring and are at greater risk for bone damage.

Conclusion to Bone Physiology.

Intrinsic and extrinsic communication between the bone cells is required at every step of the modeling and remodeling process. Intrinsic factors such as IL-1β and TGF-β are liberated from the osteocyte, osteoblasts, and osteoclasts in response to mechanical strain or a lack thereof and are critical for signaling bone turnover. Extrinsic factors which have systemic effects on the body tend to regulate the growth, and monitor or maintain micronutrient homeostasis. These factors have varying effects on bone resorption and formation processes. There is a continuum of physiological responses with the net result of bone loss or perhaps bone gain. In summary, the cross talk between the basic units of bone is complex and continues to be examined.

Quantitative Ultrasound

The quantitative ultrasound (QUS) technique uses two measures (broadband ultrasound attenuation (BUA) and speed of sound (SOS)) to estimate the quality and quantity of the bone. The Achilles Insight device utilizes solid-state transducers which allow for a real-time image to view proper placement of the heel (Damilakis, Papadokostakis, Perisinakis, Maris, Karantanas, 2007). This image allows for black and white space to be observed within a region of interest area, identified with a red circle. The region of interest is to be placed over the white area and the view can be increased so that primarily white area is in the region of interest. BUA consists of the loss of energy from the initial ultrasound beam as it proceeds through the tissue to the second transducer (Paakkunainen, Raittinen, Viikari, Seppanen, Simell, 2002). Specifically, it is the transit time by the width crossed, expressed as m/s (Gonnelli & Cepollaro, 2002). SOS accounts for the velocity of the ultrasound waves as they pass through tissue to the other transducer (Paakkunainen et al., 2002). The equation for SOS is "$SOS = (E/r)^{1/2}$ where E is the modulus of elasticity and r is the bone density expressed in g/cm^3" (Gonnelli & Cepollaro, 2002). The Os Calcis Stiffness Index (OCSI) is a calculated index of bone quantity and quality based on an equation provided by GE that manufactures QUS devices such as the Achilles Insight. The equation is listed below:

$$\text{Stiffness} = (0.67 \times \text{BUA}) + (0.28 \times \text{SOS}) - 420$$

The Achilles Insight device uses a closed-water system to provide dynamic coupling utilizing either a gel or alcohol as the coupling agent. This differs from previous models where the heel was immersed in a bath of water for assessment (Damilakis et al., 2007).

When the calcaneus is measured using the Achilles Insight device, a clean foot is placed in the device. At this time, it is sprayed with rubbing alcohol that acts as the conductor for the sound waves. Previous versions of the QUS device utilized gel but it was determined that the rubbing alcohol is also an appropriate coupling agent. Compared to gel, rubbing alcohol is easier to administer, inexpensive, and does not require the foot to be cleaned after the measurement. Due to the low cost of the Achilles Insight, and short duration of time for measurement reducing the motion artifacts that were present in previous QUS devices, it has been suggested as a tool in everyday clinical practice (Damilakis, et al., 2007; Cepollaro et al., 2005).

The validity of the Achilles Insight has been explored by observing the incidence of fracture in an elderly population based on the OCSI values produced. The Achilles Insight has been shown to be effective at discriminating between those that have experienced a hip fracture and those that have not. All ultrasound variables (BUA, SOS, and OCSI) were lower in the fractured group. Although, BMD measurements of the hip at the greatest discriminatory ability to detect differences between groups. The coefficients of variation were 0.4% for BUA, 3.0% for SOS, and 2.1%

for OCSI on the Achilles Insight device (Cepollaro, Gonnelli, Montagnani, Caffarelli, Cadirni, Martini, & Nuti, 2005). In an elderly population followed for two years, BUA decreased significantly. Therefore, Zochling and colleagues (2004) suggest that BUA is useful in longitudinal studies (Zochling, Nguyen, March, Sambrook, 2004). Therefore, it appears that the Achilles Insight is capable of detecting differences between fracture groups and presumably, exercise training groups.

The majority of studies report measuring one foot (rather than both feet) for establishing OCSI values (Wang, Moore, Crawford, Hudes, Sabry, Marus, & Bachrach, 1999; Arnett & Lutz, 2002; Daly, et al., 1997; Paakkunainen et al., 2002; Lehtonen-Veromaa, Mottonen, Nuotio, Heinonen, & Viikari, 2000; Cvijetic et al., 2003). The determination of which foot is appropriate to measure however is controversial. For example, Wang et al. evaluated the left-side, (Wang et al., 1999) while Arnett and Lutz evaluated the right side (Arnett & Lutz, 2002), both without providing reasoning.

In a study comparing gymnasts to controls along with their mothers, QUS values were measured for both feet in the children while the mothers left foot was only measured. After it was established that the means were similar, the left foot was evaluated for all participants (Nurmi-Lawton et al., 2004).

In a different study evaluating male gymnasts versus normally active controls, limb dominance was decided through questioning of

kicking leg by the investigator (Daly et al., 1997). This kicking leg method was also imposed when evaluating nutritional impacts on bone in prepubescent children (Paakkunainen et al., 2002). Lehtonen-Veromaa and colleagues (2000) measured the nondominant heel, which was based upon what hand dominance they concluded with further research that 92.4% of right-handed subjects were also right-footed (Lehtonen-Veromaa et al., 2000). Cvijetic et al. evaluated the non-dominant side, however they did not provide a rationale or procedure for establishing the non-dominant foot (Cvijetic et al., 2003). Oral, Tarakci, & Disci evaluated the dominant vs. non dominant foot using a paired t-test. However it was not established how dominance was decided (Oral et al., 2006). Bayer and Kutilek (1997) and Oral and colleagues (2004) concluded that both heel measurements should be taken and the lowest measurement should be utilized or that one side should be decided upon (Bayer & Kutilek, 1997; Oral, Yaliman, & Sindel, 2004).

Overall, it appears that a mean of both feet can be appropriate since there is so much disagreement in the literature regarding which foot is more appropriate to measure. These values are highly correlated as indicated by Trimpou and colleagues (2010) where a correlation of the left and right heel t-scores was 0.90, $p<0.0001$ (Trimpou, Bosaeus, Bengtsson, Landin-Wilhelmsen, 2010). Additionally, follow-up studies have been limited and thus the tracking of ultrasound changes is currently necessary.

Correlation between DXA and QUS.

Dual-energy X-ray absorptiometry (DXA) at certain sites has been shown to have significant correlations with quantitative ultrasound (QUS). A correlation of 0.88 was shown between BUA and DXA at the os calcis when testing with the Achilles Lunar device (Schott, Hans, Sornay-Rendu, Delmas, & Meunier, 1993). In the Achilles Lunar device, SOS and DXA correlated with an r = 0.86. This information however led these authors to conclude that there is a possibility that the same bone properties are not being measured by the two devices (Schott et al., 1993). With the Achilles Insight device, the correlations have not been shown to be as high as other QUS devices. However, the correlation between the stiffness index and BMD was high, ($r=0.75$; $p<0.0001$). The correlation of BUA and SOS with BMD were also high, ($r=0.76$ and $r=0.69$, respectively) (Damilakis et al., 2007). In a Chinese population, these values were not as high; however BUA and SOS were not reported. The correlations of OCSI and BMD at the spine, femoral neck and total hip were 0.458, 0.562, and 0.583, respectively. The confidence interval for osteoporosis of the Achilles Insight device was significant, (CI =0.861-1.005) (Jin, Lin, Zhang, & Chen, 2010). Therefore, it still appears that the Achilles Insight device is specific enough to determine those in the osteoporotic category.

It has been established that DXA measures somewhat different properties than QUS. DXA measures bone mineral mass and density while QUS measures factors related to bone strength and geometric

aspects. For example, SOS, indicates features of bone elasticity and strength. Also, it was shown that SOS is primarily sensitive to changes in external forces such as exercise and not maturation (Nurmi-Lawton et al., 2004). Therefore, DXA and QUS seem to be measuring different bone properties, in which both are important.

In another study comparing the DXA with QUS, the authors concluded that there was a highly significant correlation between QUS and bone mineral density (BMD) ($r = 0.44 – 0.70$). The stiffness index values had an even higher correlation with BMD than SOS or BUA ($r = 0.60$, 0.56, 0.58 respectively). BUA values correlated with age ($r = 0.34 – 0.54$), height ($r = 0.32 – 0.56$) and weight ($r = 0.59 – 0.60$) at the same level as DXA, whereas SI and SOS were not as highly correlated (no r-value listed) (Sundberg, Gardsell, Johnell, Ornstein, & Sernbo, 1998).

In a cross-sectional study, gymnasts, runners, and non-athletic controls were evaluated. In all of the participants studied, the calcaneal BUA and SOS values correlated significantly ($r = 0.53$ and 0.45, respectively) with the BMD of the femoral neck in the runners and in controls, but not in the gymnasts. While physical activity correlated weakly ($r = 0.19$, $r = 0.35$), but significantly with mean BMD and QUS values respectively in pubertal subjects. The authors therefore claim that physical activity accounted for more of the variation in the DXA values than the QUS values (Lehtonen-Veromaa et al., 2000).

QUS data have also been significantly correlated with BMC measurements taken at the femoral neck, greater trochanter, and lumbar spine both at baseline and at follow-up (Arnett & Lutz, 2002). These authors concluded that OCSI measurements are highly related to the bone mineral content of the lumbar spine, femoral neck, and greater trochanter (Arnett & Lutz, 2002).

Wunsche et al. (2000) found that during childhood and adolescence (6-18 years), in males and females, QUS bone densitometry is sensitive for detecting small changes of BMD that occur due to disease. QUS is also capable of detecting growth phases and pubertal changes (Wunsche, Wunsche, Fahnrich, Mentzel, Vogt, Abendroth et al., 2000).

Jaworski and colleagues (1995) discovered that BUA had the highest correlation to BMD, also proving that DXA and QUS can display similar relationships if performed properly (Jaworski et al., 1995). The correlation between BMD and QUS was stronger in children than in adults, which allows QUS to be used particularly in children. BUA also correlated to stiffness and SOS. As expected, osteopenic children defined by DXA were significantly lower than in healthy controls (Jaworski et al., 1995).

In a study comparing DXA to QUS, the stiffness index had a higher correlation with BMD than BUA or SOS which was contrary to the study mentioned previously correlating BUA with BMD. BUA did correlate with age, height, and weight which was the same results as the DXA however.

The stiffness index and SOS correlated with these same factors of age, height, and weight also, just at a lower level. Peak bone mass was established based upon the DXA measurements occurring at fifteen years in females and eighteen to twenty in males. Unfortunately, QUS was reported to not be capable of establishing peak bone mass values (Sundberg et al., 1998). Fricke et al. (2005) found that body height had the strongest influence on SOS values. There was also a dependence of SOS on age (Fricke, Tutlewski, Schwahn, & Schoenau, 2005).

In a study of young and middle-age to older women, there was a strong relationship in all women between age and the right and left stiffness index (SI) values ($p<0.001$). Additionally, significant relationships existed between the lumbar spine, femoral neck, left femur BMDs and the right and left SI except for left SI and lumbar spine BMD. These correlations ranged from 0.32-0.40 in young women, increasing in the middle-aged to older women to 0.43-0.61. In all women, these values ranged from 0.54-0.68 (Iida, Chikamura, Aoi, Ikeda, Matsuda, Oguri, Onno, Katada, Ishizaki, 2010). Therefore, it appears that in women as a whole, the correlations between measurements were significant and the machines appear to be measuring similar bone characteristics.

In a postmenopausal population, QUS and DXA were performed yearly for seven years. The femoral neck, ward's triangle, trochanter and lumbar spine $L_2 - L_4$ BMD and BMC were measured by DXA. The correlations between BUA, SOS, and SI to BMD and BMC values were

significant throughout the study along with the changes in these values (r = 0.20-0.53) except at the proximal radius and between the femoral neck BMC and SOS. Also, it appeared that a QUS t-score of less than -3.65 was the equivalent to a -2.5 t-score by the DXA for diagnosing osteoporosis. Therefore, the ultrasound can be used for screening and when these correlations are established and the matched t-score is made, QUS can be utilized for the diagnosis of osteoporosis (Trimpou et al., 2010).

These results provide evidence that QUS devices are sensitive enough to detect the changes that occur during bone development. Measures of QUS have been significantly correlated to measures of DXA in multiple populations and therefore appear to be measuring similar bone characteristics. Therefore, QUS may be an appropriate indicator of more acute bone changes than DXA.

Biochemical Measures

Bone formation is caused by osteoblastic activity. Osteocalcin (bone gamma-carboxyglutamic acid (Gla)-protein), is synthesized by osteoblasts and is the most abundant non-collagenous bone protein and the seventh most prominent protein within the human body (Lian & Gundberg, 1988). Approximately 80% of the content of mature bones is made up of osteocalcin. Only small amounts of osteocalcin are not bound to the bone matrix and thus are available for measurement in serum as a marker of bone formation (Weber, 2001). Studies have shown that serum

measures of bone GLA-protein can be used to detect differences in those with bone diseases when compared to controls. Bone GLA-protein is specific for determining differences between patients with various diseases (i.e., Paget's disease of bone, bone metastases, primary hyperparathyroidism, and osteopenia) (Price, Parthemore, Deftos, & Nishimoto, 1980). There are several radioimmunoassays (RIA) for serum osteocalcin; having varying degrees of sensitivity. Homologous RIA testing was found to be less sensitive than heterologous RIA or two-site immunoradiometric assays in a healthy female population (Minisola, Rosso, Romagnoli, D'erasmo, Manfredi, Damiani, De antoni, & Mazzuoli, 1997). Further research exploring different serum osteocalcin assays compared eight different commercial assays and determined that all provided acceptable results for healthy populations; however they concluded that values from different assay kits should not be compared across studies utilizing different assay kits (Masters, Jones, Purves, Cooper, & Cooney, 1994).

Factors affecting the circulation of serum osteocalcin levels.

A variety of factors such as age, diurnal variations, racial differences, renal clearance, endocrine effects on serum osteocalcin, PTH, calcitonin, pregnancy, menstrual and luteal phase, exogenous corticosteroids, growth hormone and thyroid hormone all play a role in determining circulating serum osteocalcin levels at a given time. Other

factors have been shown not to influence the circulating osteocalcin levels including: the follicular phase, the use of oral contraceptives, sleep, season, or usual nonsteroid anti-inflammatory drugs (Kanbur, Derman, Sen, Kinik, 2002; Nielsen, 1994). For the purposes of this review, only the factors such as variations in age, diurnal variations, racial differences, exogenous corticosteroids, PTH and growth and thyroid hormones, and exercise, that are pivotal for understanding the role of osteocalcin with osteoporosis will be discussed.

Age.

Aging and growth greatly affect osteocalcin levels. It is accepted that osteocalcin levels will vary during growth. During the neonatal period, 20-40 ng/mL is common. After this, a drop-off results until the onset of puberty where the range is between 10 and 25 ng/mL. During puberty, values of 40-80 ng/mL have been reported in males. However, after puberty and through the fourth decade, values remain between 2-12 ng/mL. These ranges are reported in Table 1. Correlations have been shown relating osteocalcin levels to age in those 25-75 years (Minisola et al., 1997).

Table 1

Ranges Of Osteocalcin Levels By Age

Stage	**Osteocalcin Level**
Neonatal	20-40 ng/mL
Onset of Puberty	10-25 ng/mL
Puberty	40-80 ng/mL
Post-puberty-4th decade	2-12 ng/mL

In boys divided by the five Tanner stages, serum osteocalcin levels were significantly higher in stage two than in one and three. Serum osteocalcin levels were highest in the Tanner stage four boys verses all other stages. In boys, serum osteocalcin appears to rise at age twelve, peak approximately two years later, and then decline to "adult levels" by age 15-16 years (Kanbur et al., 2002). Differently from the boys, in a study of girls by Tanner breast stage, osteocalcin was maximal in stages two and three, and decreased significantly in stages four and five to adult values (Blumsohn, Hannon, Wrate, Barton, Al-Dehaimi, Colwell, & Eastell, 1994).

In a study of children's osteocalcin levels, in boys GLA protein decreased significantly at age 15 and continued to decrease to adult levels by age 16. In girls, this effect was observed at the end of the 12th year and reached adult female values by age 14 (Cioffi, Molinari, Gazzerro, Di Finizio, Fratta, Deufemia, & Puca, 1997). These findings were similar to those displayed by Low and Lau (1992), indicating a peak at 12-14 years in girls with a significant decrease by age 14-16. In contrast, the boys peaked at 12-14 years, then continued to further reduced levels at 16-20 years (Low & Lau, 1992). Contrary to these findings, Bouillon and colleagues (1992) observed the highest serum osteocalcin values between 10-12 years in girls and 14-16 years in boys, indicating a slightly earlier peak in the girls (Bouillon, Vanderschueren, Van Herck, Nielsen, Bex, Heyns, Van Baelen, 1992).

In the NHANES cohort, women in the 50-79 year age group had significantly greater serum osteocalcin levels than the 20-49 year group. Additionally, men had higher levels in the 20-49 year group but in the 50-79 year age group, women had significantly higher levels than the men, displaying the shift in circulating serum osteocalcin levels with age by gender (Gundberg, Looker, Nieman, Calvo, 2002). Additionally, in a study comparing postmenopausal women, those 59 years and older had significantly higher serum osteocalcin values than the 49-59 year old group. Only the 59 year and older group displayed a significant relationship with age. These data indicate that serum osteocalcin continues to increase later in life in women (Lumachi, Ermani, Camozzi, Tombolan, & Luisetto, 2009). However, in men, no differences were observed when compared to values of younger adults (Bouillon et al., 1992).

In regard to menopausal state specifically, Yasui and colleagues (2006) displayed that serum osteocalcin was significantly higher in one-year post menopausal women than in pre- or peri-menopausal women. The pre- and peri-menopausal women had no significant differences in their serum osteocalcin levels (Yasui, Uemura, Tomita, Miyatani, Yamada, Miura, & Irahara, 2006).

Similarly, a study comparing serum levels to the iliac crest cortical samples obtained from subjects immediately postmortem showed similar differences by gender. Women's serum osteocalcin levels appear to peak

in the sixth decade of life and men's in the eighth decade. Later in life, these levels decrease. This study also demonstrated that the actual bone osteocalcin were consistent with the serum osteocalcin measurements and therefore do reflect osteoblast levels (Vanderschueren, Gevers, Raymaekers, Devos, & Dequeker, 1990).

The biological variations of serum osteocalcin have been studied in a population of 4-65 year olds. Before puberty, there is a strong correlation between serum osteocalcin and body weight, height, and chronological age. Serum osteocalcin was higher in men than in women until age 49 and significantly higher until age 44. It significantly increased in the 50-54 year old premenopausal women when compared to those in the 45-49 year age category. Further, this study showed no effects of menstrual status, oral contraceptive use, or NSAIDS on serum osteocalcin levels (Tarallo, Henny, Fournier, Siest, 1990).

Diurnal variations.

Levels of serum osteocalcin vary throughout the day and are independent of changes in serum calcium and phosphorus. The levels appear to be lowest in the morning, reaching their lowest point around noon (Gundberg, Markowitz, Mizruchi, Rosen, 1985; Nielsen, 1994), and rise to their maximum amount between 4 a.m. and 8 a.m. (Gundberg et al., 1985). Therefore, it is necessary to control the time of day when sampling is completed.

Racial differences.

In healthy Caucasian and African American men and women, age 20-35, the response of a variety of bone related variables were studied. The average osteocalcin was significantly lower in the African Americans than in the Caucasians regardless of gender. Both groups responded to vitamin D administration similarly; both groups experienced similar significant increases in osteocalcin (Bell, Greene, Epstein, Oexmann, Shaw, & Shary, 1985).

In the NHANES cohort, non-Hispanic black men had significantly lower mean serum osteocalcin levels than the non-Hispanic white men or the Mexican Americans in the 50-79 year age group, but no difference existed in the younger age group. In women, like in men, there were no significant differences by ethnicity in the 20-49 year age group but the non-Hispanic black women had significantly lower levels than the Mexican American women (Gundberg et al., 2002).

Parathyroid, growth and thyroid hormones.

In a rat model, when the hindquarters of adult male rates were administered PTH (1-84) (parathyroid hormone) or the synthetic PTH (1-34), it was found that both acutely suppress the release of serum bone GLA-protein (Calvo, Fryer, Laakso, Nissenson, Price, Murray, & Heath, 1985). This study was repeated with the 1-34 synthetic PTH in healthy adult women and similarly, bone GLA protein significantly decreased.

This thereby validated the use of serum bone GLA protein as a measure of bone formation (Riggs, Tsai, Mann, 1986).

In a study comparing children with growth hormone deficiencies to those with these deficiencies that were receiving treatment to healthy controls, those undergoing treatment did not have different serum levels of bone GLA-protein. However, the children that were not treated had significantly less serum bone GLA-protein (Delmas, Chatelain, Malavai, & Bonne, 1986). In a group of deficient children, when growth hormone treatment was administered, serum osteocalcin levels increased significantly at six and twelve months when compared with the baseline values (Low & Lau, 1992). Therefore, it appears that with treatment, serum osteocalcin levels can be improved in children with growth hormone deficiencies.

Normal adult males were studied for the seven day treatment effects of recombinant human growth hormone (HGH) on markers of bone turnover and BMC. In the treated group, serum bone GLA-protein increased significantly during treatment and remained elevated for six months. Thus, the authors concluded that the administration of recombinant HGH stimulates osteoblasts and activates bone remodeling (Brixen, Nielsen, Mosekilde, & Flyvbjerg, 1990). Excess thyroid hormone causes accelerated bone growth and development in children.

Exogenous corticosteroids.

In an in vitro study of the effects of the administration of two different glucocorticoids: prednisone and deflazacort, and of parathyroid hormone similar responses were observed on serum osteocalcin. Once the glucucorticoids or parathyroid hormone were administered, osteocalcin production was inhibited in a dose-dependent manner (Beresford, Gallagher, Poser, & Russell, 1984).

In a double-blind trial comparing the administration of 40 mg of prednisone for five days to a placebo, serum bone GLA-protein was significantly decreased by 75% in the prednisone group. Since, there was no matched reduction in bone turnover, the authors concluded that short-term glucocorticoid treatment will result in a negative bone balance (Nielsen, Thomsen, Eriksen, Charles, Storm, & Mosekilde, 1988).

Similarly, in glucorticoid-treated asthmatics, serum osteocalcin levels were significantly less (50%) less than in asthmatic controls (Reid, Chapman, Fraser, Davies, Surus, Meyer, Huq, & Ibbertson, 1986). In rheumatoid arthritis and other forms of arthritides, serum osteocalcin levels were also lower than the healthy age-matched controls. After the administration of prednisone (20 mg/d), serum osteocalcin significantly decreased but NSAIDS had no effect on serum osteocalcin (Ekenstam, Ljunghall, Hallgren, 1986). Therefore exogenous corticosteroids appear to significantly reduce serum osteocalcin levels, thus negatively affecting bone balance.

Exercise

In a study comparing the differences in serum osteocalcin between athletes and non-athletes, the response of serum osteocalcin to exercise in these groups was also analyzed. At baseline, athletes had significantly higher serum osteocalcin levels than non-athletes. Immediately following 30 minutes of exercise on a treadmill, the non-athletic group's serum osteocalcin significantly increased but 60 minutes post exercise decreased back towards baseline values. In the athletic group, osteocalcin slightly decreased immediately after exercise and increased significantly from baseline, 60 minutes following exercise. At 30 minutes there were no differences between groups, however at 60 minutes following exercise, the athletic group was significantly higher than the non-athletic group (Nishiyama, Tomoeda, Ohta, Higuchi, & Matsuda, 1988).

In a study of the effects of marathon running in noncompetitive athletes, subject's serum osteocalcin levels were measured ten days before, immediately after the marathon, and one, three, and five days after their run. In men and women differences were observed. Serum osteocalcin dropped immediately following the run, but not significantly. One day following the run and for three days after, men's serum osteocalcin levels was significantly lower. In women, this significant decrease in serum osteocalcin levels remained for the five day follow-up (Malm, Ronni-Sivula, Viinikka, & Ylikorkala, 1993).

Weightlessness has the opposite effect of exercise on bone. In a study evaluating bone metabolism during simulated spaceflight in rats, serum osteocalcin values were significantly less than in control rats (Patterson-Buckendahl, Grindeland, Martin, Cann, & Arnaud, 1985). Therefore, it appears that serum osteocalcin levels remain higher in active, athletic populations and are reduced in those undergoing weightlessness.

A cross-sectional study comparing endurance runners to age-matched controls on BMD and markers of bone turnover was analyzed. The runners that were training an average of seven hours per week had significantly higher BMD, but their serum osteocalcin was lower than the controls by 10.7% although this was not significant (Brahm et al., 1997). This study contradicts those previously mentioned studies by Nishiyama et al. 1988, Malm et al. 1993, and Patterson-Buckendahl et al. 1985. It is possible that since the study is cross-sectional in nature, other confounding factors were not controlled for in this study leading to the contradictory results.

Conclusion.

A variety of factors have been studied for their effects on serum osteocalcin. It appears that the commercially available kits are appropriate for measuring serum osteocalcin, but comparisons in values should not be made between different kits. Additionally, special care needs to be taken to screen for conditions affecting PTH, growth hormone, thyroid hormone, those taking corticosteroids, and exercise history. These

factors can drastically affect the baseline levels of participants and their response to varying treatments. Serum osteocalcin does appear to be a viable tool to measure the bone formation taking place within the body.

Weight Bearing Activity

Although substantial research has taken place in regard to the types of physical activities that can be successfully utilized to increase bone mass in youth or maintain bone mass in older women, there has been no "ideal" training program developed that has been successful across these populations (Hughes, Novotny, Wetzsteon, & Petit, 2007). Therefore, it remains important to identify the most feasible, effective mode of increasing bone, specifically during adolescence when peak bone mass can be maximized. This overview will focus on the previous research programs for pre- and pubertal children. The overview will be to describe what could be considered the "ideal" training program for those in the pre- and pubertal stages.

In humans, most bone mass is thought to be achieved during the pubertal growth spurt, particularly Tanner stages two through four (Kohrt, Bloomfield, Little, Melson, & Yingling, 2004). In addition, the original work of Frost who studied the effects of loading, made it clear that a torque must be applied to the bone in order for it to continue to adapt, turnover and become stronger (Frost, 2000). Taken together and knowing that lean body mass levels are considered the strongest predictor of BMD in women (Ilich-Ernst et al., 2002), it is reasonable to understand why it is

important to focus the prevention interventions on both muscle and bone at this critical development period.

Training Load.

The 2004 ACSM Position Stand on Physical Activity and Bone Health provides a thorough summary of the training modes considered to be most effective by specific populations. For all populations, it appears that applying a greater number of loading cycles each day is more beneficial than applying these loads during a single exercise session. Therefore, there is speculation that the longer the recovery period between sets or bouts of exercise, a greater bone response can be evoked.

ACSM Recommendations.

Physical activities that involve impact such as jumping, gymnastics, plyometrics and resistance training, as well as sport participation that involves running and jumping seem to be the most beneficial for bone health. Since the force applied to the bone is what ultimately increases mass, applying a greater load provides a proportional increase. Ground reaction forces have been measured to be three to eight times body weight when performing jumping activities and can be ten to fifteen times body weight when performing gymnastics maneuvers (McKay et al., 2005a, Kohrt et al., 2004). Whereas, when walking the ground reaction forces are only one to two times body weight (Kohrt et al., 2004). Thus, it seems logical to focus on training programs that involve the greatest, safest imposable force to the body. A load of no more than 60% 1RM is

recommended for this age group in regard to resistance training (Kohrt et al., 2004). Thirty to sixty minutes are recommended for a combination of these activities that target all muscle groups. It seems that a training program that involves some form of resistance training via calisthenics, machines, or free-weights in combination with jumping and dynamic movements would be the most effective in this pubertal population. Therefore, the circuit training model incorporating both resistance and cardio portions may prove to be the best training concept to date to elicit the greatest osteogenic effects. It is important to note however, that this amount of training seems counterintuitive given the data that support intermittent activities for the maximum osteogenic effect and given the data of the "Bounce at the Bell" program using only three bouts of ten jumps throughout the school day (McKay et al., 2005a).

These recommendations described below are for the attainment of a high peak bone mass and are not for optimal health in general. For children to achieve optimal health, the recommendations of the U.S. Department of Health and Human Services for children to attain 60 minutes of moderate intensity exercise per day, seven days per week include 60 minutes of weight-bearing, dynamic activity at least three days per week.

Frequency: A minimum of three days per week should be recommended for increasing bone parameters during growth. Intervention studies that have been effective at increasing bone parameters range from three to five

days per week of training (Arnett & Lutz, 2002; Fuchs et al., 2001; Gilsanz, Wren, Sanchez, Dorey, Judex, & Rubin, 2006; Gunter et al., 2008a; Gunter et al., 2008b; Johannsen et al., 2003; MacKelvie, McKay, Khan, & Crocker, 2001; MacKelvie et al., 2003; McKay et al., 2005a; Nichols et al., 2001; Weeks et al., 2008). Training should follow a progressive undulating (with peaks and points of reduced intensity training) cycle to allow for recovery and allow for continuous adaptations to be made (Baechle & Earle, 2000).

Intensity: High-intensity training should be utilized. This training should emphasize high forces being applied to the body with jumping. Countermovement jumps (repeated jumps without a break in jumping, where one load is placed by jumping up and a different load is absorbed by the bones during the landing), as was used by McKay and colleagues (2005) in their school-based intervention are effective. It is important to recognize that the intensity and load placed upon the body will differ with the style of jumping used. Plyometric jumps dropping from a 10cm box and jumping upon landing were shown to have the highest ground reaction forces (5.5) followed by countermovement jumps (5.3) and jumping jacks (3.5) (McKay et al., 2005b). Therefore, different types of jumping should be taught in the schools to be utilized in a jumping program. Since jumping cannot target all sites related to osteoporotic fracture, resistance training in a traditional or non-traditional setting should also be emphasized. Although ACSM recommends no more than

60% of 1RM for children to improve bone, the National Strength and Conditioning Association (NSCA) position stand on youth resistance training recommends starting children with a light to moderate load (50-70%) of one to two sets. Following this introduction, with proper training, the child can utilize heavy loads with less repetitions (60-80% for intermediate; 70-85% for advanced) (Faigenbaum, Kraemer, Blimkie, Jeffreys, Micheli, Nitka, & Rowland, 2009). It is understood that the higher the load, the greater the force placed upon the body. Therefore, teaching children to lift appropriately will enhance their ability to load these other skeletal sites that cannot be targeted effectively with jumping.

Time: First, it seems that the greatest osteogenic response is achieved by spreading activity out into multiple bouts throughout the day rather than one concentrated session. This also makes performing the exercise feasible as a large amount of time does not need to be allotted in order to perform the exercise. The literature supports a minimum of three sessions as short as it takes to complete 10 jumps to be performed throughout the day (McKay et al., 2005a). When adding resistance training to the program, it is reasonable to expect a longer time requirement, but none of the programs shown to be effective lasted longer than 45 minutes. Therefore, it seems reasonable that for most days of the week only a few minutes is necessary to perform jumping. On the days that resistance training is used, 30 minutes of activity may be required.

Type: Since the body is also continuously adapting, it is important to change the type of training regularly. Jumping seems to be the most feasible mechanism for increasing bone parameters during growth as no equipment is needed. Jumping should not be isolated to simple two-footed jumps, rather changed weekly to a variety of jumping applications: one-footed jumping, two-footed jumping, jumping side to side, front to back, plyometric jumping utilizing boxes, and performing jumping patterns as has been utilized as a training mechanism for sports with dot training (jumping from different "dots" on the floor in a given pattern to provide for a dynamic workout). These styles of jumping should be rotated during the training period in order to overload the body.

Sport.

Cross-sectional studies of sport participants are a common mode of analysis in the research literature as a means of comparing active adolescents to their less active peers. While cross-sectional studies have limitations for interpreting causal influence, they can provide some insight on the effects of sport participation on bone health. In a comparison of competitive jump ropers, soccer players, and age-matched inactive young women, Pettersson and colleagues (2000) demonstrated significantly greater BMD and bone area in jump ropers compared with soccer players and controls, with soccer players also superseding the controls (Pettersson et al., 2000). This research was conducted on girls during adolescence after they had participated in their sport for an average of six years. Thus,

clearly they had been training through the critical period known to increase bone mass (Kohrt et al., 2004). Zanker et al (2003) exhibited a higher total body BMD in pre-pubescent girls that participated in gymnastics for at least two years versus controls (Zanker, Gannon, Cooke, Gee, Oldroyd, & Truscott, 2003). This improvement prior to the onset of puberty, in theory should result in maintained higher BMD values in adolescence.

Jumping.

Arnett and Lutz (2002) compared the differences in quantitative ultrasound stiffness index values between a typical PE class, and classes where students incorporated five minutes or ten minutes of jump roping into the class time. The authors showed that jump roping for ten minutes increased bone strength measured by QUS, displaying the greatest increase in bone strength over the control group of 'typical PE students.' The five minute jump roping group also had significantly higher QUS values than controls (Arnett & Lutz, 2002).

Witzke and Snow (2000) have also shown a trend towards increases in BMC of those participating in a plyometric jump training protocol for 30-45 minutes, three days per week (Witzke & Snow, 2000). In the Bounce at the Bell program, ten countermovement jumps, three times per day during the school day improved BMC over controls (McKay et al., 2005a). This provides possibly the strongest argument for a feasible, short, simple approach to increase bone parameters and maximize the

osteogenic effect. By spreading small amounts of jumping out to three different times of day as was done in the Bounce at the Bell program, the osteogenic effect appears to be high and effective. A summary of the programs that have been effectively utilized to increase bone mineral content and density with jumping and other interventions is included in Table 2.

Table 2

Training Studies

Authors	Duration	Days/Wk	Type	Bone changes
Arnett & Lutz, 2002	5-10 minutes	4	Basic jumps	OCSI
Fuchs, Bauer, Snow, 2001	100 jumps	3	61cm box jumps	FN, LS BMC, LS BMD
Gilsanz et al., 2006	10 minutes	7	Vibration training	Spine vBMD
Gunter et al., 2008	90-100 jumps	3	Basic jumps	LS, FN, TH, WB BMC
Gunter et al., 2008	20 minutes	3	Box jumps	Hip BMC
Johannsen et al., 2003	25 jumps	5	45cm box jumps	WB BMC
MacKelvie et al., 2001	10 minutes	3	All jumps	FN, LS BMC
MacKelvie et al., 2003	10 minutes	3	All jumps	FN, LS BMC
McKay et al., 2005	30 jumps	5	Countermovement jumps	FF, IR BMC
Nichols, Sanborn, Love, 2001	30-45 minutes	3	15 Resistance Exercises	FN BMD
Weeks, Young, Beck, 2008	10 minutes	2	All jumps	FN, LS BMC

Table 2. An overview of studies performed to increase BMC, BMD or OCSI in children. Duration is described in number of jumps reported or minutes spent in activity. The type of activity is described generally as the authors described it. Bone changes reported were all significant. OCSI: Os Calcis Stiffness Index, FN: Femoral Neck, LS: Lumbar Spine, vBMD: Volumetric Bone Mineral Density, BMC: Bone Mineral Content, BMD: Bone Mineral Density, TH: Total Hip, WB: Whole Body, FF: Proximal Femur, IR: Intertrochanteric Region

Table 2 displays that a range of frequencies, types, and durations of training have been successfully utilized to improve bone parameters. It appears that three days per week is the most common training regimen

used and that 10-100 jumps is the minimum amount of jumps necessary to produce these significant improvements.

Other Training Modalities.

Other viable methods for increasing bone mass are resistance training and/or vibration training. The only trial to test vibration in healthy adolescents showed improved vBMD (volumetric BMD) of the spine over controls (Gilsanz et al., 2006). Resistance training is used primarily in conjunction with jumping programs in children. However, one study evaluated the effects of resistance training alone in adolescent females and showed significant improvements over controls in Wards triangle and femoral neck aBMD (areal BMD) (Nichols et al., 2001). It seems that further research is needed isolating resistance training and vibration training as mechanisms for improving bone parameters in healthy children.

Conclusion.

Utilizing training at least three days per week for a minimum of a few minutes each day broken into various sessions seems to be ideal for providing the maximum osteogenic effect. Jumping is feasible, effective, and can be done without equipment. In order to target other sites than the femoral neck and spine, it is likely necessary to utilize resistance training that specifically targets the other sites. Special considerations should be made for the child's ability and coordination when instituting new programs. As previously mentioned, an undulating training cycle should

be utilized to prevent injuries and maximize adaptations. Short-duration jumping programs throughout the day, at least three days per week should be encouraged for bone health.

Introduction to Nutrition

The nutritional recommendations made for prevention of osteoporosis range throughout the lifespan. Since osteoporosis is a pediatric disease with geriatric consequences, it is imperative to develop the highest possible peak bone mass during adolescence/young adulthood. From a nutritional standpoint, prevention of bone loss is specific for various age groups. The primary nutrients that are the focus of the prevention of osteoporosis are calcium, vitamin D, and vitamin K. Other nutrients have been studied for their effects on bone health but will not be the focus of this discussion. These nutrients are: phosphates, protein, sodium, and vitamins A and C.

According to the newest edition of The Institute of Medicine's Dietary Reference Intakes (DRIs) (2010) the recommendations for calcium and vitamin D is dependent upon age. For adolescent girls (9-18 years), 1300 mg/day with an upper limit of 3000 mg/day is recommended. In young adult women (ages 19-50), the recommended calcium dietary allowance is 1000 mg/day with an upper limit of 2500 mg/day. The recommended dietary allowance for 9-50 year olds for Vitamin D is 600 IU/day with an upper limit of 4000 IU/day (Institute of Medicine, 2010). Vitamin K has not been re-addressed since 2001. The

recommendation for vitamin K is 60 μg/day for 9-13 year olds, 75 μg/day for 14-18 year olds, and 90 μg/day for women ages 19 and up (Panel on Micronutrients, et al., 2001).

The following discussion will address the need for each of these nutrients for bone health and the potential benefits of supplementation for improvements in nutrient and/or bone status.

Calcium

Bone contains about 99% of the body's calcium (Heaney, 2009; Lanham-New, 2008). Calcium has two primary roles: supporting structural integrity and regulating metabolic function (Lanham-New, 2008). Calcium is so important to many functions of the body, including muscle contraction and nerve transmissions, that extracellular fluid calcium concentrations must be tightly controlled within a very narrow range (Heaney, 2009). Serum calcium is regulated by calciotrophic hormones including: parathyroid hormone (PTH), 1,25 dihydroxycholecalciferol, and calcitonin. Serum calcium is also influenced by sex hormones, growth hormones, corticosteroids and other locally-acting hormones (Lanham-New, 2008). Calcium is lost through the shedding of skin, hair, nails, sweat, and urinary and fecal excretions. When calcium absorption does not meet calcium losses, blood calcium decreases can result in a chain reaction of events, including the secretion of parathyroid hormone (PTH), which results in resorption of bone into the blood (Heaney, 2000). It is important to note that once adequate

calcium intake is reached, the consumption of additional calcium results in no greater benefits (Heaney, 2009).

Food sources.

Dairy products provide more calcium per calorie than any other food in the diet. Dairy also provides other necessary nutrients necessary for bone metabolism including protein, magnesium, potassium, zinc and phosphorus (Heaney, 2000). Other food sources of calcium include cooked spinach, turnip greens, broccoli, bok choy, collard greens, mustard greens, and kale. Sardines with bones, canned salmon with bones, tofu, oranges and some breads also contain calcium (Miller, 1989; Whitney, Rolfes, 2008). Soy products are another source of calcium however the calcium in soy beverages is reported to be less available than dairy calcium within the body (Heaney, 2009).

The Dietary Guidelines for Americans (2005) reported that seven of seven randomized controlled trials reported a positive relationship between dairy consumption and BMC and/or BMD. Of the observational studies, 25/32 reported this positive relationship between dairy consumption and BMC and/or BMD (Dietary Guidelines Advisory Committee, 2005).

The relationship of calcium intake to bone parameters.

When trying to establish relationships between calcium intake, physical activity, and bone status in 5-19 year old children, average calcium intake was positively related to size-adjusted average bone

mineral content (BMC) in girls and boys. However, the accretion of size-adjusted average BMC after tracking these children for one-year did not result in differences by average calcium intake or physical activity (Molgaard, Thomsen, & Michaelsen, 2001). Therefore, although mean calcium intake was related to bone status, this did not persist during the one-year of bone tracking. Contrary to these findings, VandenBergh and colleagues (1995) found no relationship in 7-11 year old children between mean calcium intake and BMC measures. This group emphasized that the only differences in BMC values were dependent upon physical activity status alone (VandenBergh, DeMan, Witteman, Hofman, Trouerbach, & Grobbee, 1995).

In a study of men and women who entered the study in their youth (9-18 years), and completed in their 20's (20-29 years), the relationship of calcium intake and bone mineral density (BMD) was established. Regardless of calcium intake, no differences were observed in lumbar spine BMD when adjusting for weight. However, in women, calcium intake was correlated with femoral neck BMD in all age groups. When these age groups were combined, mean calcium intake was significantly related to their femoral neck BMD. There is a ceiling effect; these authors noted that there were no further increases of femoral neck BMD with calcium intakes greater than 1200 mg/day (Valimaki, Karkkainen, Lamberg-Allardt, Laitinen, Alhava, Heikkinen, Impivaara, Makela, Palmgren, Seppanen, Vuori, & the Cardiovascular Risk in Young Finns

Study Group, 1994). It appears that calcium intake is related to bone status, however once adequate intake is reached, no further bone improvements are attained.

Supplementation.

In a review of 52 controlled trials it was concluded that 50 studies reported improved bone parameters including reduced bone remodeling, improved calcium retention, and reduced age-related bone loss or fracture risk by increasing calcium intake through supplementation. Ranges of dosage were 35-40 mmol/day for growth and 22-40 mmol/day for mature adults. Of 86 observational studies, 64 reported improved bone parameters with increased calcium intake in the diet. The consensus of this review was that 75-80% of studies reported that increased calcium intake via the diet or supplementation resulted in positive bone changes, with the remaining studies reporting null findings (Heaney, 2000).

Sadideen and Swaminathan (2004) studied the overnight effects of an acute oral calcium load of 400 mg on serum PTH and bone resorption as measured by s-CTX. This group concluded that this dose decreased bone resorption and serum PTH (Sadideen & Swaminathan, 2004). Other studies that utilized 1000 mg calcium loads were also effective at decreasing bone resorption and serum PTH (Scopacasa, Horowitz, Wishart, Need, Morris, Wittert, & Nordin, 1998; Zikan et al., 2001). However, the 1000 mg load can result in renal stones or gastrointestinal tract disturbances due to the high single exposure. Calcium

recommendations are for 1000 mg or more per day, but it is not suggested that this be consumed in one dose. Therefore, Sadideen and Swaminathan (2004) suggest that it is possible that a 400 mg calcium load is sufficient and safer than 1000 mg loads to reduce bone resorption and serum PTH in both younger and older adults.

In prepubertal girls, calcium fortified foods (from milk extract) were used as the form of supplementation in a randomized controlled trial for one year. The calcium supplemented group experienced greater BMD gains at the radial and femoral sites. These gains were greatest in the subset of the supplemented group that had a spontaneous calcium intake below the median intake of 880 mg/day. BMC and bone area also were greater in the supplemented group. A relationship was also shown between the cumulative amount of calcium consumed from the calcium-enriched foods and mean BMD at the six skeletal sites measured (Bonjour, et al., 1997). In 10-year old girls, calcium-fortified milk, calcium plus vitamin D fortified milk, or no supplementation were provided among different schools for two years. Both supplemented groups improved their size-corrected BMD over the control group, but the majority of improvements were observed in the lower limbs (Zhu et al., 2008). This specific change in the lower limbs would suggest that some sort of loading could have also promoted this observed effect.

Dairy products were used as the supplementation source to observe changes in body composition and bone parameters, among girls in Tanner

Stage 2. Baseline calcium intake was not reported but was reported as not significantly different between groups. Supplementation with dairy products for 12 months was utilized to get the dairy group to consume at least 1200 mg/day of calcium intake. This intervention resulted in the supplemented group experiencing greater gains (non-significant) in lumbar spine and total body BMD despite no changes in body composition. It is important to note that with dairy consumption, phosphorus, vitamin D and protein intakes also increased in this group and all nutrients were associated with the increase in BMD observed. Therefore, it is not necessarily a product of the calcium intake alone that increased the BMD of the supplemented girls (Chan, Hoffman, & McMurry, 1995). A comparison of pill-supplementation of 1000 mg/day of calcium plus vitamin D3 of 200 IU/day, calcium alone (1000 mg/day), cheese (1000 mg/day calcium), or placebo was conducted in Tanner Stage I and II girls age 10-12. In this analysis, girls supplemented with cheese had a significantly higher percentage change in cortical thickness than all other groups. Total body BMD was also significantly higher in the cheese group than in the placebo group. These statistically significantly improvements in the cheese group were not maintained once growth velocity was accounted for in the analysis (Cheng, Lyytikainen, Kroger, Lamberg-Allardt, Alen, & Koistinen, 2005).

Similarly, Cadogan and colleagues (1997) reported improvements in lumbar spine and total body BMD and BMC when adding 300 mL of milk

to the intervention group's diet for 18-months. The 12-year old girls were also not meeting recommendations for calcium intake at baseline (739-753 mg/day). Due to the use of milk as the supplementation, it also was not clear that calcium was the source for bone improvements. Protein, phosphorus, magnesium and zinc intakes all increased in the supplemented group. Regardless, greater bone acquisition resulted from 300 mL of additional milk per day (Cadogan, Eastell, Jones, & Barker, 1997).

In 14-year old girls, at least one year postmenarcheal, with low calcium intakes (<800 mg/day), 1000 mg/day of calcium carbonate was supplemented for one-year in a randomized controlled trial. The supplement significantly reduced bone turnover and serum PTH. Accordingly, those in the supplemented group accrued significantly more total body BMD and increased their percentage of lumbar spine BMD accretion compared to controls. However, the supplemented group was non-significantly higher in percentage change in femoral neck BMD (Rozen et al., 2003).

Collegiate female athletes were supplemented with 1000 mg/d of calcium citrate with 400 IU of vitamin D and compared to a placebo group for 16 weeks (during their competitive season). Those supplemented with calcium and vitamin D averaged above recommended levels of calcium and small, but insignificant increases in leg BMD (0.8%) were observed. When the basketball players were analyzed as a sub-group, they displayed

a significant 1.5% increase in total body BMD over the 16-week study (Mehlenbeck, Ward, Klesges, & Vukadinovich, 2004).

In long-distance runners supplemented with 1000 mg/day of calcium carbonate for one year, these findings were not repeated. Although bone mineral loss was prevented at the femoral mid-shaft, no differences were observed in hip or spine BMD. The authors suggested that calcium intake has a greater effect on the cortical bone (measured at the femoral mid-shaft) than the trabecular bone measured at the other sites (Winters-Stone, Snow, 2004). It may be important to note that the mean calcium intake for subjects was already >1000 mg/day of calcium at baseline therefore the additional 1000 mg/day may have resulted in no greater benefit based on previous findings (Valimaki et al., 1994).

Soy products have also been used and recommended as calcium sources for those who do not eat animal products but their effectiveness is controversial. Different preparations of soy products are available, both with and without isoflavones, a substance believed to act with estrogen on the bone. The effects of soy protein with isoflavones and soy protein without isoflavones were studied in healthy 20-25 year old women for one year. However, neither group improved their BMD or BMC over the course of the study despite other factors such as menstrual status and body composition remaining constant (Anderson, Chen, Boass, Symons, Kohlmeier, Renner, & Garner, 2002).

There are essentially two calcium sources used to fortify soymilk by manufacturers: tricalcium phosphate (TCP) and calcium carbonate (Zhao, Martin, & Weaver, 2005). In a study of the absorbability of calcium from soy milk, TCP was shown to seemingly be less absorbable than calcium from cow's milk (Heaney, Dowell, Rafferty, & Bierman, 2000). As a follow-up to this study, TCP was compared to calcium carbonate as additions to soy milk and cow's milk. Zhao et al. (2005) found that the fractional absorption of calcium did not differ between cow's milk and calcium carbonate supplemented soy milk, but both were significantly higher than TCP-supplemented soy milk, emphasizing the importance of calcium carbonate as the most effective calcium source supplemented in soy milk (Zhao et al., 2005).

It is important to note that with all forms of calcium supplementation, serum 25(OH)D status can affect calcium absorption. In a study evaluating the absorption rates of calcium, in those pre-treated with 20 μg of 25(OH)D compared to no pre-treatment of 25(OH)D, absorption of calcium was 65% higher at elevated serum 25(OH)D levels (Heaney, Dowell, Hale, Bendich, 2003). Therefore, the benefits of calcium supplementation can be dependent upon serum 25(OH)D status.

Dairy verses calcium carbonate.

The absorption of calcium from milk, calcium carbonate, and calcium carbonate plus 600 IU of vitamin D were compared. There were no differences in urinary calcium excretion between persons given milk

and calcium carbonate supplementation throughout the day (3 tablets), however calcium carbonate plus vitamin D resulted in greater urinary calcium excretion. The authors concluded that there are no differences in the rate of calcium absorption from milk versus calcium carbonate supplements but that with the addition of vitamin D, healthy women can increase their bioavailability of calcium (Mortensen & Charles, 1996).

Contrary to these findings, a study comparing milk to calcium sulfate and calcium carbonate for two and a half months in two-month old pigs resulted in significant differences in bone strength and mineralization despite similar bone size and weight (Pointillart, Coxam, Seve, Colin, Lacroix, & Gueguen, 2000). Therefore, it is possible that despite the similar calcium bioavailability of milk and calcium carbonate, the bone mineralization, and thereby strength, is comparably different.

A rat study comparing dairy versus calcium carbonate for its ability to increase peak bone mass and on bone maintenance during a subsequent calcium deficiency displayed the advantages of dairy. The rats in the dairy group improved the strength and size of the bone and maintained that strength and size when they were placed into a calcium depleted state when compared to the calcium carbonate group (Weaver, Janle, Martin, Browne, Guiden, Lachicik, & Lee, 2009).

In a study of 10-12 year old girls with a mean calcium intake of 664-680 mg/day at baseline, those supplemented for one year with 1000 mg/day of calcium from low-fat cheese had a greater improved cortical

thickness of the tibia compared to the placebo, calcium supplemented (1000 mg/day) or calcium plus vitamin D supplemented (1000mg/day Ca + 200 IU/day vitamin D) groups. The cheese supplemented group also had higher BMD compared to the placebo group with a compliance of >50% in the cheese group (Cheng et al., 2005).

Bone mass accrual (BMC and bone area) appear to be maintained in those supplemented with foods fortified with milk-extracted calcium (three-year follow-up) (Bonjour, Chevalley, Ammann, Slosman, Rizzoli, 2001) whereas those receiving calcium carbonate lost those improvements in BMC and BMD within 12 months to three years following the intervention (Lee, Leung, Leung, Wang, Xu, Zeng, & Cheng, 1997; Slemenda, Peacock, Hui, Zhou, & Johnston, 1997).

This prolonged effect was previously studied by Fehily and colleagues (1992). In a 14-year follow-up of subjects that participated in a milk-supplementation trial at ages 7-9, the subjects, now aged 20-23 years, had slightly higher BMC and BMD measurements than the control group. Although this effect was non-significant, there are a variety of other factors that played a role in the subjects' BMC and BMD, including the participant's body weight, alcohol consumption, current calcium and vitamin D intake, and sport participation during adolescence that all had relationships with BMD or BMC (Fehily, Coles, Evans, & Elwood, 1992).

Recommendations.

Dairy seems to be the greatest enhancement for bones because it is a rich source of calcium, phosphorous, magnesium, potassium, and protein. Milk may be the best product for a person to be able to obtain all dietary components necessary for increased bone mass rather than calcium supplementation alone. Holbrook and Barrett-Connor (1991) have suggested that milk should be utilized in the diet when available (Holbrook & Barrett-Connor, 1991). Weaver and Mobley suggest that individuals would benefit from the three servings of dairy products recommended by the Dietary Guidelines for Americans (Weaver & Mobley, 2007; Dietary Guidelines Advisory Committee, 2005). The newest recommendation suggests at least 1300 mg/day for adolescent girls and 1000 mg/day for young adult women (Institute of Medicine, 2010).

Soy products can be used to replace cow's milk when necessary if fortified with calcium carbonate (Zhao et al., 2005), however calcium carbonate supplementation has been shown to lose its bone benefiting effects following the termination of supplementation (Bonjour et al., 2001; Lee et al., 1997; Slemenda et al., 1997). Therefore, recommending a soy-based replacement for dairy products may not confer the greatest long-term bone benefits.

Vitamin D

The importance of Vitamin D was brought to the forefront by the industrial revolution that led to many children developing the condition of

rickets, a bone-deforming condition. Children were not able to go outside due to the pollution which resulted in an estimated 80-90% of children living in Leiden, The Netherlands and Boston, Massachusetts with this condition (Holick, 2008; Holick, 2003a; Holick, 1994). The resolution to this condition was first cod liver oil, then later on with milk fortification with vitamin D (Holick, 2006). However, rickets has re-emerged in recent years due to a variety of reasons such as lower milk and dairy consumption and reduced sun exposure (Papandreou, Malindretos, Karabouta, & Rousso, 2010).

Vitamin D comes primarily in a plant version (ergocalciferol) and animal version (cholecalciferol): vitamin D_2 and D_3, respectively. Vitamin D_3 has been demonstrated to be far more effective for increasing serum 25(OH)D markers than D_2 (Papandreou et al., 2010; Armas, Hollis, Heaney, 2004; Trang, Cole, Rubin, Pierratos, Siu, Vieth, 1998; Houghton & Vieth, 2006). Armas and colleagues (2004) reported that the potency of D_2 is less than one-third of D_3 (Armas et al., 2004). Similarly, Trang et al. (1998) displayed a 1.7 times greater efficacy of D_3 than D_2 (Trang et al., 1998). Therefore, for the purposes of extracting the appropriate recommendations for vitamin D supplementation, only studies evaluating the effects of vitamin D_3 will be utilized.

The best tool for the measurement of vitamin D status is the serum 25(OH)D level because it measures both the vitamin D synthesized by the skin and the vitamin D ingested through the diet (Zitterman, 2003;

Papandreou et al., 2010). The use of this tool is recognized as the standard by the Institute of Medicine (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997).

In regard to bone health, vitamin D works to maintain blood concentrations of calcium and phosphorus to strengthen the bone. This happens in three ways: enhanced absorption of calcium and phosphorus from the GI tract, reabsorption of these minerals by the kidneys, and mobilization from the bones to the blood. With enough of these circulating minerals, bones are able to become more dense and stronger (Whitney, Rolfes, 2008). The active form of vitamin D is calcitriol and is classified as a hormone within the body. The vitamin D (cholecalciferol) that is consumed from the diet or synthesized in the skin is activated in the liver and kidneys, forming calcitriol, and also affects many metabolic functions, including calcium absorption in the small intestine (Bergman, Gray-Scott, Chen, & Meacham, 2009).

Vitamin D deficiency.

When adequate vitamin D status is not achieved, the small intestine is only able to absorb half of the dietary calcium that can be absorbed under sufficient conditions (Holick, 2004b). The usual 30% net intestinal calcium absorption is reduced to only 10-15% gross absorption (Heaney et al., 2003; Gennari, Merlott, De Paola, Matini, & Nuti, 2009). A deficiency of vitamin D can also result in thin, brittle, soft, or misshapen bones as occurs in the condition of rickets in children and osteomalacia in adults

(Bergman, et al., 2009; Holick, 2004b; Holick, 2006). However, it is "one of the most common undiagnosed medical conditions in the world" (Holick, 2008). It is accepted that a vitamin D deficiency is a serum concentration of 25(OH)D <20 ng/mL, insufficiency is 21-29 ng/mL and adequacy is greater than 30 ng/mL. "Adequacy" of vitamin D status will allow for intestinal calcium absorption to remain efficient (Holick, 2008; Holick 2006; Holick 2007; Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Norman, Bouillon, Whiting, Vieth, & Lips, 2007). Based on these criteria, it is estimated that one billion people are in a vitamin D deficient or insufficient state (Holick, 2008; Holick, 2006; Holick 2007). In the Boston Medical Center study, 36% of the 18-29 year olds were vitamin D deficient at the end of winter (Tangpricha, Pearce, Chen, & Holick, 2002). In Northern Ireland, 50% of the adult population studied had insufficient serum 25(OH)D at baseline, with the other subjects closely approaching the insufficient range (Barnes, Robson, Bonham, Strain, & Wallace, 2006). Cashman and colleagues (2008) also demonstrated in their vitamin D supplementation study that the summer skin exposure to sun was not sufficient to maintain minimum serum 25(OH)D levels in a 20-40 year old population in Northern Ireland (Cashman, Hill, Lucey, Taylor, Seamans, Muldowney, FitzGerald, Flynn, Barnes, Horigan, Bonham, Duffy, Strain, Wallace, & Kiely, 2008). However, in Israeli adolescents, even for those in the lowest tertiles of vitamin D status, physical activity was able to elevate BMD values over

those of their less active peers. This physical activity effect was not seen in those that were vitamin D sufficient (Constantini, Dubnov-Raz, Chodick, Rozen, Giladi, & Ish-Shalom, 2010).

Food sources.

Vitamin D is a nutrient that occurs in limited food sources. It is present in fatty fish such as salmon and canned tuna, cod liver oil, irradiated mushrooms, and egg yolks (Bergman et al., 2009; Bordelon, Ghetu, & Langan, 2009; Holick, 2004a; Holick, 2004b). Some foods in the United States are fortified with the nutrient including milk, orange juice, and cereal (Bordelon et al., 2009; Holick, 2004a; Holick 2004b).

Sun exposure.

Approximately 90% of vitamin D requirements are met through sun exposure but this is dependent upon a variety of factors (Holick, 2004a; Holick, 2007; Norman et al., 2007; Holick, 2003a; Holick, 2003b). Factors that affect vitamin D synthesis include: season, latitude, time of day, cloud cover, smog, clothing and the use of sunscreen (Bergman et al., 2009; Holick, 2004a; Holick 2004b; Holick, 2003b; Holick, 1994; Webb, Kline, & Holick, 1988; Holick, 2007). For those living above or below the 37° N latitude line, this will determine at what times of year they will be able to synthesize adequate vitamin D. Those living above this North latitude line will experience a substantial decrease in vitamin D produced in the skin from November to February. It is important to note, however, that since vitamin D_3 is fat soluble, it can be stored in the body fat and

used during the winter months (Holick, 2004a; Holick 2004b; Holick, 2007). Even during spring, summer, and fall months it is necessary to go outside for 5-15 minutes between 10 am and 3 pm to receive enough photons to produce D_3 (Holick, 2008). In addition, Matsuoka and colleagues (1987) displayed in vivo that sunscreen applied 1-hour before sun exposure blocked all synthesis of vitamin D resulting in no serum 25(OH)D changes when compared to those not applying sunscreen (Matsuoka, Ide, Wortsman, MacLaughlin, & Holick, 1987). This study highlights the importance of allowing the skin exposure to direct sunlight each day.

The most important times of day for sun exposure (10 am – 3 pm) are a vital concern as most adults who work inside are not outside during these hours. Therefore, they are unable to expose their skin to the UVB rays for their body to synthesize vitamin D. This was shown in a study at the Boston Medical Center. Approximately one-third of those working at the Medical Center between the ages of 18-29 years were vitamin D deficient by the end of the winter. This also was determined not to be dependent upon milk intake. However, those who took multi-vitamins during summer and winter months had higher levels of 25-hydroxyvitamin D levels (Tangpricha et al., 2002). Therefore, it appears that supplementation with a multi-vitamin increases serum 25(OH)D levels over those not supplementing with a multi-vitamin. However, without the

sun exposure during the critical time of day for exposure, workers appear to be predisposed to becoming vitamin D deficient by the winter months.

Since the majority of vitamin D is thought to be received via sunlight, cross-sectional studies tend to infer that a greater serum vitamin D status is indicative of higher sun exposure. In an analysis of the 1998-2004 NHANES data, young white adults in the highest quartile of serum 25-hydroxy vitamin D levels had 4.1% higher bone mineral density than those in the lowest quartile. There was a significant positive relationship between bone mineral density and serum 25-hydroxy vitamin D levels for all categories (young and older whites, Mexican-Americans, and blacks).

Contrary to this finding, in a study of post-pubertal females aged 16-22 years living in California, no significant relationships existed for 25(OH)D status and bone mineral density measurements at any site. 59% of subjects were in the insufficient range, however none were in the deficient category possibly leading to these differing results (Kremer, Campbell, Reinhardt, & Gilsanz, 2009).

In a study of 18-70 year old participants comparing tanning bed users to non-users, those who used a tanning bed at least one day per week had significantly higher mean 25(OH)D serum levels than non-tanners. The tanners also had significantly higher BMD and z-scores at the total hip than non-tanners. No differences existed between groups at the spine or total body BMD (Tangpricha, Turner, Spina, Decastro, Chen, & Holick, 2004).

Sun exposure appears to be the most cost-effective and efficient means to prevent vitamin D deficiency if environmental conditions permit (Holick, 2003a; Holick, 2003b).

Supplementation.

Supplementation studies have primarily been conducted in older populations. The studies that have provided supplementation to subjects that are still able to increase their bone mass will be highlighted here.

In a randomized, double-blind 12-month intervention examining a healthy Danish Caucasian population of 11-12 year old girls, the dose-related effects of supplementation (5 and 10 μg/day) were evaluated on serum 25(OH)D status and whole body and lumbar spine bone mass (Molgaard, Larnkjaer, Cashman, Lamberg-Allardt, Jakobsen, & Michaelsen, 2010). The only significant differences between the supplemented groups and the non-supplemented group at 6- and 12-months were significantly greater serum 25(OH)D and lower lumbar bone area with the lowest area in the 10 μg/day group when compared to controls. No differences existed between groups for size-adjusted BMC of the total body and spine (Molgaard et al., 2010).

Similarly, in 11-year old Finnish girls, a placebo, 5 μg and 10 μg were given daily to subjects for one-year. However, no differences were observed in the femur and vertebrae BMD, BMC or bone area (BA) between groups. Once the change in BA, weight, and Tanner stage were added as covariates to the model, and 80% compliance was met,

significant differences were observed. BMC in the femur was significantly higher in the supplemented groups but only lumbar spine BMC was improved versus controls in the 10 μg group (Viljakainen, Natri, Karkkainen, Huttunen, Palssa, Jakobsen, Cashman, Molgaard, & Lamberg-Allardt, 2006).

In 18-28 year old Finnish men in the military, serum 25(OH)D concentration was analyzed for a baseline of one year. These men were then given vitamin D fortified margarine and milk which increased their wintertime serum 25(OH)D by 50% and overall decreased their prevalence of vitamin D insufficiency by 50% during this same time frame (Laaksi, Ruohola, Ylikomi, Auvinen, Haataja, Pihlajamaki, & Tuohimaa, 2006).

The effects of a 22-week, 4-group vitamin D_3 supplementation randomized trial at two sites in Ireland were evaluated by Cashman et al. (2008). Twenty to forty year old participants were randomized to receive a placebo (0 μg/day), 5 μg/day, 10 μg/day, or 15 μg/day vitamin D supplement. Serum 25(OH)D increased in a dose-response relationship with supplementation as would be expected. There was no significant effect of this supplementation on serum albumin-corrected total calcium concentration however PTH concentrations were significantly lower in the group receiving the 15 μg verses the placebo group (Cashman et al., 2008).

Barnes and colleagues designed an eight-week, double-blind randomized intervention in 18-27 year old males and females to determine if vitamin D supplementation would affect circulating total calcium and

PTH levels. However, the 600 IU/day did not result in differences between the supplemented and non-supplemented groups for serum total calcium levels or PTH (Barnes et al., 2006).

When studying overweight and obese subjects, mean baseline serum 25(OH)D levels were in a sufficient range. Despite the high supplementation of vitamin D (40,000 IU/wk and 20,000 IU/wk) for one year, no changes were observed in BMD (although none would be expected since most subjects fell in the normal range at the start of the study). Also, it is important to note that serum total calcium values did not change in the supplemented group although serum PTH did decrease in these high supplemented groups. No serious adverse effects of the supplementation were reported (Jorde, Sneve, Torjesen, Figenschau, Hansen, Grimnes, 2010).

In an adult population, (19 men and 36 postmenopausal women ranging from 33-78 years), supplementation of 500 IU/day of vitamin D and 500 mg/day of calcium for seven months resulted in significant improvements in lumbar and femoral BMD compared to the control group that continued to lose bone (Meier, Woitge, Witte, Lemmer, & Seibel, 2004). This study emphasizes the importance of vitamin D in maintaining or improving bone parameters.

Recommendations.

Although the newest recommendations for vitamin D intake are 600 IU/day for young women (Institute of Medicine, 2010), Bischoff-

Ferrari and colleagues (2006) suggested that to reach desired mean concentrations of serum 25(OH)D, 700-1000 IU/day are required. The upper limit of the recommendation is currently set at 4000 IU/day and this is the recommendation by this same group for reaching high (beneficial) 25(OH)D ranges (Bischoff-Ferrari et al., 2006). Other research groups, however, have recommended lower levels of vitamin D supplementation. Cashman et al. (2008) recommended at least 41 μg/day (164 IU/day) to maintain serum vitamin D levels in the sufficient range. However, these researchers also noted that the results of their analysis could be estimating a low required value. Heaney and colleagues (2003) reported a minimum of 114 μg/day to elicit the same serum levels of 25(OH)D (Heaney, Davies, Chen, Holick, Barger-Lux, 2003). Therefore, it appears that a minimum of 600 IU/day is necessary to remain sufficient. When and where sun exposure is available, 5-15 minutes should be spent outdoors exposing the arms and legs to the sun. Higher levels should be recommended to ensure that during winter months and times where sun exposure is not available that each person will remain sufficient.

Vitamin K

Vitamin K is necessary for blood clotting but it also is involved with the synthesis of bone proteins. Vitamin K functions to act in γ-carboxylation of glutamate resides to form γ-carboxyglutamate (Gla) in proteins that are dependent on Vitamin K such as osteocalcin. The Gla

residues have a high affinity for calcium and thus are the reason for its focus in regard to skeletal health (Morgan, 2009).

In order for calcium to bind to form normal bones, to decrease bone turnover and to protect against hip fractures, vitamin K is required. Vitamin K is created in the GI tract by the bacteria that reside there. Once it has been synthesized, it is absorbed and stored in the liver (Whitney & Rolfes, 2008). Vitamin K can also be ingested via the diet and/or supplements. Approximately 90% of the vitamin K stored in the liver as menaquinones (vitamin K_2) (Shearer & Newman, 2008; Shearer, Bach, & Kohlmeier, 1996). Other organs also have some storage capacity (Shearer, 2009). In a study of the vitamin K turnover, regardless of the dosage, an oral dose of phylloquinone (vitamin K_1) resulted in losses of 20% through the urine and 40-50% through the feces (Shearer, McBurney, & Barkhan, 1974). Therefore, approximately 60-70% of vitamin K consumed is excreted from the body within a few days indicating the body's ability to constantly replenish vitamin K stores (Shearer et al., 1996). There is no single biomarker of vitamin K status, therefore multiple biomarkers are utilized. However, serum uncarboxylated osteocalcin is considered to be a "sensitive indicator of the vitamin K status of bone" (Shea & Booth, 2008).

In the Framingham Offspring cohort, bone mineral density at the femoral neck, trochanter, ward's area, and spine were significantly different between the lowest and highest quartiles of phylloquinone intake (ranging from a mean intake of 70 μg/day in the low group to 309 μg/day

in the high intake group) in women. This remained true when the population was divided into those less than or greater than 59 years. However, this difference was not observed in the men (Booth, Broe, Gagnon, Tucker, Hannan, McLean, Dawson-Hughes, Wilson, Cupples, & Kiel, 2003).

Food sources.

Approximately half of the vitamin K necessary is received from food and the remaining is a result of bacterial synthesis in the GI tract. Phylloquinone (vitamin K_1), is the primary dietary form of vitamin K found in green leafy vegetables. Menaquinones (vitamin K_2) have different structures and can be found in low doses in animal-based foods such as chicken meat and some cheeses. It can also found in large amounts of legumes, including fermented soybeans (Shea & Booth, 2008).

Vitamin K is available in green vegetables including: broccoli tops, kale, lettuce, parsley, collards, spinach, bib lettuce, brussel sprouts, and cabbage. It is also highly available in vegetable oils including soybean oil and canola oil. Other foods containing lower doses of vitamin K (10-100 μg) are French beans, red cabbage, cauliflower, chick peas, cucumber, olive oil and peas (Whitney & Rolfes, 2008; Shearer et al., 1996).

In a study of phylloquinone intake in a British population, 60% came from vegetables, 19-23% came from cooked vegetables including cabbage, broccoli, brussel sprouts, and spinach, and 14-18% by raw salad vegetables (Thane, Bolton-Smith, & Coward, 2006). However, in a US

population, 40-50% of intake came from these green leafy vegetables (Booth, Pennington, & Sadowski, 1996).

Supplementation.

In elite female young adult long-distance runners, vitamin K_1 supplementation (10 mg/day) for one month was studied for its effects on calcium binding capacity of osteocalcin to determine if greater positive balance could be achieved between resorption and bone formation. In the amenorrhoeic group, vitamin K supplementation increased bone formation markers by 15-20% and decreased bone resorption markers by 20-25%. In the oral contraceptive user group, supplementation did not result in such improved markers of bone formation and resorption but resorption was affected more than formation in this group (Craciun, Wolf, Knapen, Brouns, & Vermeer, 1998).

In pre- and peri-menopausal women, six-months of vitamin K_1 supplementation was administered at a dose of 600 μg/day. This resulted in no improvement of BMD or biochemical indices of bone formation or resorption when compared to controls. A high attrition rate with the lowest BMD subjects dropping out of the study, could have affected the results (Volpe, Leung, & Giordano, 2008).

When comparing younger (18-30 years) to older women (65+ years), baseline serum phylloquinone levels were lower in the younger population. In both groups, with 1000 μg/day vitamin K_1 for two weeks, the mean under-γ-carboxylated osteocalcin percentage decreased

significantly without differences by age or sex. However, other measures of bone turnover (N-telopeptides of type I collagen (NTx) or bone specific alkaline phosphatase (BSAP)) remained unchanged. Therefore, the authors concluded that osteocalcin may be the first biomarker of bone turnover that changes as a result of vitamin K supplementation (Binkley, Krueger, Engelke, Foley, & Suttie, 2000).

Recommendations.

It appears that the national recommendations of 90 μg/day vitamin K is likely sufficient for bone metabolism. Although higher intake levels have been associated with higher BMD values in the Framingham Offspring Cohort and 1000 μg/day vitamin K had advantageous effects on osteocalcin levels in women, long-term studies of these effects are still needed (Booth et al., 2003; Binkley et al., 2000). Until better markers of vitamin K are established and its role is better understood, a minimum of 90 μg/day appears to be appropriate for healthy adult women (Panel on Micronutrients et al., 2001).

Protein

Protein composes approximately 50% of the volume of bone and 33% of its mass. Its effects on bone health have been credited as both beneficial and detrimental but this is dependent upon factors including: the level of protein in the diet, dietary protein source, and calcium intake. Protein affects the bone in 4 ways: 1) provides the structural matrix of the bone, 2) optimizes serum IGF-1 levels, 3) reportedly increases urinary

calcium, and 4) reportedly increases intestinal calcium absorption (Heaney & Layman, 2008). It is commonly accepted that there is a positive relationship with protein intake and urinary calcium. In a study where calcium intake was set at 20 mmol/day and protein intake was increased from 0.7 g/kg to 2.1g/kg, intestinal calcium absorption significantly increased. However, the long-term effects of this increase in calcium losses, and increases in absorption are not yet understood (Kerstetter, O'Brien, & Insogna, 2003).

In cross-sectional studies, the association of milk, meat, and total dietary protein has been associated with BMD. In adolescent girls, milk protein intake was positively associated with size-adjusted BMC at the trochanter and lumbar spine after controlling for calcium and total energy intakes and physical activity. Although total protein intake also was positively associated with BMC, this association did not remain after controlling for calcium, total energy intakes, and physical activity. Therefore, milk protein intake appears to be associated with higher BMC (Budek, Hoppe, Ingstrup, Michaelsen, Bugel, Molgaard, 2007).

In young women, when calcium intake was low and protein intake was high, there was a positive effect on BMD and BMC. However, when both calcium and protein intakes were high, this was reported to be detrimental to BMD and BMC (Teegarden, Lyle, McCabe, McCabe, Proulx, Michon, 1998). In another study in young women, no differences were observed between protein intake and BMD. However, women in the

lowest tertile of vegetable protein consumption had a lower BMD at the hip, spine and total body than those consuming more vegetable protein (Beasley, Ichikawa, Ange, Spangler, LaCroix, Ott, & Scholes, 2010). In a Japanese population of young women, low protein intake was associated with low BMD values. Calcium intake was also only 66% of the RDA in this lowest BMD group (Hirota, Nara, Ohguri, Manago, Hirota, 1992). It appears that in this population, low protein intake in combination with lower calcium intake results in lower BMD values. In line with this study, subjects were followed from childhood to adulthood (mean age 23 years) to determine bone mineral accrual in relation to other factors. Protein intake was a predictor of total body BMC accrual in all subjects. In subjects who had adequate calcium intake at peri-adolescence or adulthood, protein intake positively predicted total body BMD, total body BMC net gain, and total body BMD (Vatanparast, Bailey, Baxter-Jones, Whiting, 2007). Therefore, when calcium is adequate, it appears that protein intake has a positive affect on bone health.

Food sources.

It is generally accepted that dietary animal protein evokes a greater increase in urinary calcium than vegetable protein (Kerstetter et al., 2003). However this topic remains controversial. Protein intake in the US and Canada is considered to be sufficient in most people (recommended to be 0.8 g/kg). Sources of protein other than meat are milk, grains, legumes, nuts, and eggs (Whitney & Rolfes, 2008).

Supplementation.

In strength and power trained football players, three groups of protein intake were established amongst the players studied. Those consuming the lowest amount of protein (<1.2g/kg/day) had significantly elevated serum osteocalcin levels when compared to those in the moderate intake (1.2-1.9 g/kg/day) or high intake (>1.91 g/kg/day) after 10-weeks of periodized heavy resistance training. There were no relationships between consumption groups and BMD (Ratamess, Hoffman, Faigenbaum, Mangine, Falvo, Kang, 2007).

In healthy women, milk basic protein (MBP) (40 mg/day) was used as a supplement for six months. When compared to controls, bone mineral density of the left calcaneus significantly increased. Concurrently, markers of bone turnover decreased (c-NTX and deoxypyridinoline/creatinine) significantly in the supplemented group (Aoe, Toba, Yamamura, Kawakami, Yahiro, Kumegawa, Itabashi, & Takada, 2001). In this same study, radial bone mineral density also significantly increased in the supplemented group over the placebo treatment (Yamamura, Aoe, Toba, Motouri, Kawakami, Kumegawa, et al., 2002).

Similarly, in a supplement study of the same dose of BMP (40 mg/day) for six months, lumbar BMD increased significantly more in the supplemented group than in the control group. Additionally, the marker of bone resorption (NTx) was significantly reduced and the marker of bone

formation was significantly increased in the supplemented group at six months. Therefore, this supplementation appears to be effective for increasing bone formation, decreasing bone turnover and ultimately increasing BMD (Uenishi, Ishida, Toba, Aoe, Itabashi, Takada, 2007).

In a Chinese young female population, whole milk consumption (~12.5 mg MBP), milk plus MBP (12.5 mg + 40 mg), and a control group were compared for changes in BMD and markers of bone turnover. All three groups had significant increases in total BMD from baseline to eight months. However, there were no differences between groups. For bone turnover, the MBP group decreased significantly from baseline to eight months and a decrease was also observed from baseline to six months in the milk group with no change observed in the control group. No significant increase in BAP was observed in any group. When combining the two milk groups, NTx significantly decreased at three, six, and eight months from baseline. Therefore, the authors concluded that milk itself is effective in suppressing bone resorption (Zou, Lin, Xu, Xu, Ma, Li, Li, & Wang, 2009).

Recommendations.

It appears that dietary protein has beneficial effects on bone markers including IGF-1 and bone mineral density. It may be especially critical for dietary protein to be sufficient during key times in the life cycle such as during the formation of peak bone mass or during high rates of bone loss later in life (Jesudason & Clifton, 2011). Despite the controversy

in the literature, it appears that when calcium intake is sufficient, a high protein intake is not detrimental to bone health and can even enhance biochemical markers of bone formation and turnover. However, low protein intake is a predictor of lower BMD in adulthood and should therefore be avoided (Vatanparast et al., 2007).

Other Nutrients

Other factors besides calcium, vitamins D and K, and protein can have an impact on bone maintenance. Vitamins A and C, phosphorus, magnesium, and fluoride can all play a role in bone metabolism and bone health.

Vitamin A.

Vitamin A is a fat-soluble compound. Sources of vitamin A in the diet are primarily from organ meats and as various carotenoid precursors, yellow and dark green vegetables including carrots, sweet potatoes, spinach, kale and turnip greens (Morgan, 2009).

There is limited research available regarding the effects of vitamin A on bone turnover and bone health. Speculation credits over-supplementation of vitamin A with adverse effects on bone health. In a study of healthy young men, the highest available does of vitamin A available (7576 μg/day) was provided to participants for six weeks. However, this resulted in no changes in markers of bone formation or resorption (osteocalcin, bone specific alkaline phosphatase (BSAP) and N-

Telopeptide of type 1 collagen (NTx) (Kawahara, Krueger, Engelke, Harke, & Binkley, 2002).

In the third National Health and Nutrition Examination Survey, ~33% of participants had fasting serum retinyl esters (markers of vitamin A) greater than 10% of total serum vitamin A, indicating excess vitamin A. Despite this high proportion of subjects in what has been hypothesized as an unsafe level (too high), no association between these makers of vitamin A and BMD were observed at any site when controlling for any significant covariates (Ballew, Galuska, & Gillespie, 2001). Therefore, it is unclear if elevated serum vitamin A markers are deleterious for bone mass.

Morgan (2009) suggests that vitamin A and its relationship to bone may not be clear because the dietary patterns themselves may be the true link to bone health. There is currently no ideal methodology for the assessment of vitamin A. There is debate about whether fasting serum retinyl esters or serum retinol levels are more appropriate for the assessment of vitamin A status. The inconsistent results in the literature regarding vitamin A and bone health require further research and validated assessment tools (Morgan, 2009).

Vitamin C.

Vitamin C is a cofactor of collagen formation, which is the primary component of the organic matrix of bone (Weber, 1999). Vitamin C is found in many fruits and vegetables (Tucker, 2009). There is no clear beneficial effect of vitamin C alone; however, deficiency has been

associated with defective connective tissue. The recommended dietary reference intake is currently 75 mg/day and 90 mg/day for adult women and men, respectively (Panel on Micronutrients et al., 2000). The studies conducted to date have focused on the effects of vitamin C status in older populations and primarily are cross-sectional in nature (Tucker, 2009). In a follow-up study of the Framingham cohort, those in the highest tertile of vitamin C intake and supplement use were at the least risk of suffering a fracture over the 17-year follow-up when compared to those in the lowest tertile. Therefore, it may be that vitamin C can act as a protective factor in bone health (Sahni, Hannan, Gagnon, Blumberg, Cupples, Kiel, & Tucker, 2009). Although the particular daily intake of vitamin C for bone health is not clear from the literature, the current recommendations of 75 mg/day and 90 mg/day for women and men respectively (Panel on Micronutrients et al., 2000) are likely appropriate for bone health (Weber, 1999).

Phosporus.

Phosphorus, acting as carbon phosphate in the body plays a structural role in bone and is a component of hydroxyapatite. Hydroxyapatite accounts for 85% of the phosphorus in the human body (Bergman et al., 2009). Absorption of phosphorus is high, ranging from 55-80% in the intestine. Therefore, since phosphorus is available in many foods in the US and is highly absorbed in the intestine, a deficiency is rarely observed (Bonjour et al., 2009). The recommended intake of phosphorus is 700 mg/day for adult women and men (Panel on

Micronutrients, 2001). It is in milk, cheese, meat, bread, cereal, bran, eggs, nuts and fish. It is estimated, however, that 20-30% of daily intake in the U.S. is from processed foods and soft drinks (Bergman et al., 2009).

Magnesium.

Magnesium is essential for many metabolic reactions within the body but in regard to bone, it is a component of bone structure and strength. Magnesium is combined with calcium and phosphorus in the bone and over 50% of the magnesium in the body is located in skeletal mass (Bergman et al., 2009; Bonjour et al., 2009). Sources of magnesium within the diet include green vegetables, nuts, seeds, legumes, some whole grains, seafoods, and beverages including coffee, tea, cocoa and hard water. However, the consumption of other products may result in excessive renal losses of magnesium such as alcohol and loop and thiazide diuretics (Bergman et al., 2009). The recommended intake for Americans is 310-320 mg/day for adult women and 400-420 mg/day for adult men (Panel on Micronutrients, 2001).

In the rat, a 50% reduction in magnesium intake below requirements resulted in reduced serum vitamin D and serum PTH concentrations. As a possible result of these reduced biomarkers, trabecular bone mineral content gain was less and osteoclast number was higher in magnesium-deficient rats at three and six months (Rude, Gruber, Norton, Wei, Frausto, & Kilburn, 2006). With a 25% reduction in magnesium intake, serum vitamin D and PTH concentrations were also

significantly reduced. Decreased bone volume and trabecular thickness also occurred however, osteoclast number did not increase with this reduction (Rude, Gruber, Norton, Wei, Frausto, & Kilburn, 2005).

It is not clear at this time if the typical lower than recommended intakes of magnesium observed in Western diets is detrimental to humans and requires magnesium supplementation or fortification (Bonjour et al., 2009). However, it appears that significant reductions in magnesium intake results in impaired serum values of biomarkers related to bone health and increased bone turnover. Therefore, it appears that meeting the recommended intakes for magnesium should be emphasized for bone health.

Fluoride.

Fluoride is about 100% absorbable when consumed as sodium fluorosilicate in fluoridated water or sodium fluoride in toothpaste or tablets. Approximately 95% of the fluoride in the body is found in the bones and teeth and its absorption is rapid and contributes to the stability of the bone mineral matrix. The recommended intake of fluoride is 3 mg/day in women and 4 mg/day in men (Panel on Micronutrients, 2001). Beverages and water contribute a high percentage of total fluoride in the diet however marine fish, clams, lobster, crab, shrimp and tea also contribute to fluoride consumption. The water used to cook or prepare foods: such as soups, juices, powdered milk, and vegetables also add to fluoride intake. It is important to note, however, that reverse osmosis

systems tend to remove a significant amount of fluoride from the water (Bergman et al., 2009).

Conclusion To Nutrition Factors.

Calcium, vitamin D, vitamin K and protein are critical nutritional components of bone health. It appears that for calcium, dairy products are the ideal products for consumption. Dairy should be used when possible over supplementation due to the improved maintenance of bone density observed in follow-up trials comparing dairy to calcium supplement usage (Bonjour et al., 2001; Lee et al., 1997; Slemenda et al., 1997). The vitamin D necessary for proper absorption of calcium, bone maintenance, and the prevention of other diseases appears to be at least 700-1000 IU/day. For those living below the 37° North latitude line, spending at least 10-15 minutes per day outside with as much skin exposure as possible is beneficial for bone health. For those not able to go outside or living above this latitude line, supplementation should be utilized as ergocalciferol during winter months or when sunlight is not available. Although data are limited regarding vitamin K's role in bone health in a healthy, young adult population, it seems that the 90 μg/day recommendation is sufficient for bone health. When calcium is adequately consumed, protein appears to also have a beneficial effect on bone health despite its controversy in the literature. Milk basic protein has been highly studied but has shown to be effective at increasing BMD and improving markers of bone formation and turnover. A well-balanced diet that includes fruits, vegetables, and dairy

products should be recommended along with small amounts of sun exposure for bone health and the prevention of osteoporosis.

Conclusion

In order to effectively prevent the disease of osteoporosis from occurring, physical activity, nutrition, and health status need to be carefully monitored. Weight bearing activity should be recommended and specifically encouraged from 11-16 years of age when peak bone mass attainment has the greatest potential to be increased (Javaid & Cooper, 2002). Weight bearing training including dynamic activities such as jumping or weight training a minimum of three days per week appears to be beneficial for bone health (Witzke & Snow, 2000; Fuchs et al., 2001; Gunter et al., 2008a; Gunter et al., 2008b; MacKelvie et al., 2001; MacKelvie et al., 2003; Nichols et al., 2001). Calcium, vitamin D, vitamin K and protein intake also need to meet recommended levels at minimum. The consumption of dairy products appears to be the greatest method of achieving recommended levels for bone health (Holbrook & Barrett-Connor, 1991; Weaver & Mobley, 2007; Dietary Guidelines Advisory Committee, 2005). Those diagnosed with a condition known to affect bone health should be closely monitored and especially encouraged to maintain a healthy lifestyle focused around weight bearing physical activity and proper nutrition. The prevention of osteoporosis is possible if a healthy lifestyle is encouraged from pre-adolescence through adulthood.

Methods

Subjects

Subjects were recruited from the local University area via classroom announcements, word of mouth, and other flyers located on campus. Once recruited, an appointment was made for the subject to report to the Healthy Lifestyles Research Center Laboratory where the study was explained and informed consent obtained (Appendix A). Once informed consent was obtained, a medical history questionnaire was completed and a heel ultrasound was performed to determine subject eligibility based on established criteria.

Eligibility requirements were the following: a) Healthy women 20-40 years with normal 21-40 day menstrual periods and b) OCSI t-score less than 1.0. Women were excluded if: a) they were not 18-40 years old, b) had an OCSI t-score greater than 1.0, c) they reported any orthopedic problems that would prevent them from participating in the study, d) reported that they had an abnormal menstrual cycle, e) reported that they had been diagnosed with amenorrhea or eating disorders, f) reported use of drugs known to affect bone status, g) reported a condition such as diabetes or hyperparathyroidism that would affect bone status or h) reported that they were moving or leaving the area before the end of the study.

Sample Size

A power calculation to determine sample size was calculated from serum osteocalcin data. A sample population of 12 women per group was calculated as necessary to detect significant (0.05) differences between groups. This was computed for a two sample population with a required difference of 0.58 ng/ml in serum osteocalcin values.

Assuming a 20% attrition rate, a minimum of 15 subjects per group were recruited for a total sample of at least 30 participants at the start of the study.

Study Design

The intervention was a parallel group design with monthly (5 total) repeated measures of OCSI, body composition, and serum osteocalcin. Subjects were stratified by use of oral contraceptives and randomized into two groups: jump training (JT) or control (C).

Rolling Recruitment and Enrollment.

Upon IRB approval, women were screened and enrolled in the study scheme. Baseline measures were collected and the intervention began once eight volunteers were randomized until 32 volunteers were enrolled. The estimated timeline for complete recruitment and enrollment was 4 weeks (~8 subjects/week). Subjects were matched (as a pair) within their groups on mean OCSI scores (average of right and left OCSI values). Once pairs were determined, a coin was flipped to establish which subject would be in the jumping and the control group.

Table 3

Enrollment Schedule

Cohort	Start Date	Completion Date
A	18-Oct	7-Feb
B	25-Oct	14-Feb
C	15-Nov	14-Feb
D	22-Nov	21-Feb

Procedures

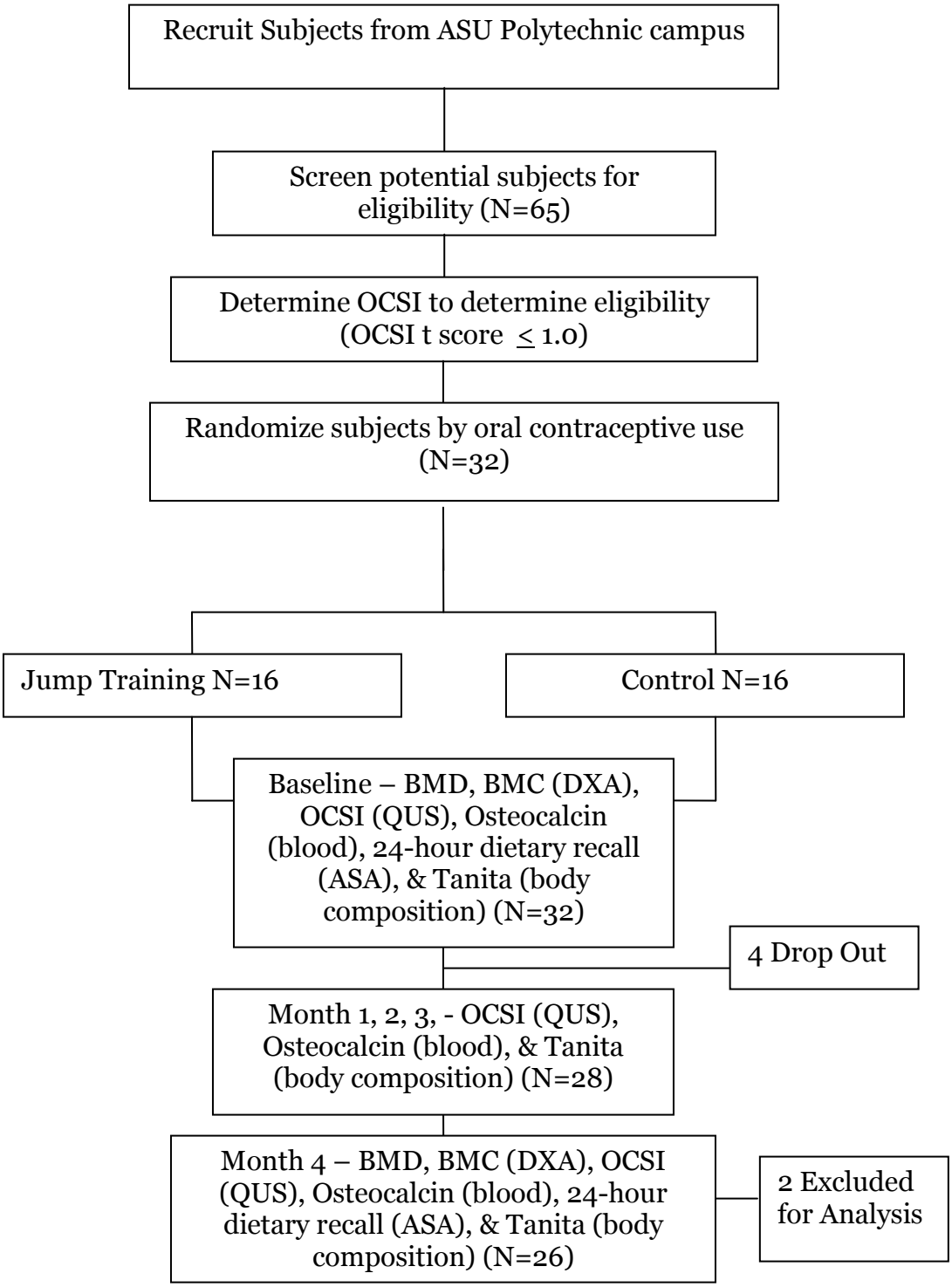
All subjects had the following measures taken at the beginning of the intervention (baseline): height using a stadiometer affixed to a wall (Seca, Ontario, California), body weight, fat mass, fat free mass, and percent fat using bioelectrical impedance analysis (Tanita, Arlington Heights, Illinois), os calcis stiffness index values using the quantitative ultrasound device (Achilles Insight, GE Lunar, Madison, Wisconsin) and blood samples ($\geq$ 20 mL) were drawn for subsequent analysis of serum osteocalcin measured at the same time each day (Human Osteocalcin RIA Kit, Stoughton, Massachusetts) (Appendix B). Total body bone mineral density and bone mineral content were assessed using dual energy x-ray absorptiometry (DXA), and a 24-hour dietary recall using an automated web program (ASA 24) was utilized at baseline and following four months of training. This cluster of tests took approximately one hour.

At three monthly time periods, subjects returned to the laboratory for Os Calcis Stiffness Index (OCSI) measures using the Achilles Insight Ultrasonometer, body composition using bioelectrical impedence (BIA),

and blood draws for subsequent analysis of serum osteocalcin. Additionally, participants were given activity logs to track physical activity performed each week and it was turned in at the end of each month when the subjects arrived for their monthly testing. Subjects in the jumping group received their training schedules for the month at their visit. Subjects returned completed physical activity logs and received new logs monthly (Figure 7).

Figure 7

Flow Chart Of Study Procedures



Self-Administered Questionnaires.

Health history questionnaire.

A health history questionnaire was utilized to screen for any diseases known to affect bone metabolism or health. Also, family history of osteoporosis, history of broken bones, and 12-month physical activity recall was included (Appendix E).

24-hour dietary recall.

Subjects were given directions to complete 24-hour dietary recalls through the National Cancer Institute (NCI) automated web-based application ASA 24 at baseline and the final week of training (ASA 24, Bethesda, Maryland) (Appendix G). The format and design of this automated computer technology is based on an interviewer-administered 24-hour recall. The ASA 24 uses graphic enhancements and an animated character to guide and probe participants to accurately assess food types and amounts. This automated program is free to researchers. The food list from which respondents select their intakes includes all foods available from USDA's Food and Nutrient Database (USDA Food and Nutrient Database for Dietary Studies, Version 4.1., Beltsville, Maryland).

Anthropometry.

Height.

Height was measured using a wall mounted stadiometer (Seca, Ontario, California). Subjects were asked to remove their shoes and stand with their heels against the platform facing out towards the room. The

average of three measures was recorded. Subjects were repositioned and re-measured each time.

Weight.

Weight was measured using a digital electronic scale (Tanita, Arlington Heights, Illinois). Subjects were asked to remove their shoes and any jewelry and step on to the scale. After each subject, alcohol wipes were used to clean the surface of the scale.

Body composition.

Body composition (fat mass, fat free mass, and percent body fat) were measured two ways:

1) BIA (Tanita, Arlington Heights, Illinois) scale. Subjects were asked to remove their shoes and any jewelry and step on to the scale. After each subject, alcohol wipes were used to clean the surface.

2) Dual Energy X-ray Absorptiometry (GE Lunar Prodigy, Madison, Wisconsin). All subjects were asked to take a quick urine pregnancy test before they could participate in any test using ionizing radiation. Subjects were asked to take off all jewelry and wear clothing with no metal. Subjects were asked to lie on a table for about 10 to 15 minutes while they received a whole body scan of low-intensity X-rays. The amount of radiation from the DXA scan is very low and the dosage has been estimated to be equivalent to 1/20th of a chest x-ray (similar to the radiation exposure of a long

airplane trip). Testing was administered by a certified radiology technician at baseline and month 4.

Heel bone stiffness.

Heel bone stiffness (Os Calcis Stiffness Index, OCSI) was measured on both feet using an Achilles Insight Ultrasonometer device (Achilles Insight, GE Lunar, Madison, Wisconsin) (Appendix F). The Achilles Insight is a quick, portable ultrasound device providing a real time image of the calcaneus. Each subject sat in a sturdy chair, placed their foot in the device, and a peg was secured between their big toe and second toe to keep the foot in place during the test. Then, the ankle was sprayed with alcohol on both sides prior to starting the assessment. Once the assessment was started, the transducers filled with water and came in contact with the foot. At this time, the real time image appeared on the screen so that the researcher could ensure proper placement of the foot. After approximately two minutes, the transducers deflated and the other foot was tested. OCSI values are established and calculated by utilizing an equation with the speed of sound and broadband ultrasound attenuation that were also measured at this time.

Biochemical indices of bone turnover.

20 ml of blood were collected and serum was prepared by the lab technician and stored at -80°C until biochemical analysis was performed. Biochemical assays included a measure of bone formation (osteocalcin). Assays were performed in duplicate, following manufacturer's directions

at ASU using the RIA Kit (Biomedical Technologies, Inc, Stoughton, Massachusetts) (Appendix B).

Jumping Intervention

The jumping group was asked to jump in their bare feet a specified number of consecutive jumps three times per week on a hard surface. Subjects were instructed to find a flat area with no objects nearby to decrease the risk of injury. Jumping was performed in bare feet in a safe location so that the body (bones) absorbed the shock instead of the shoes, which could differ between individuals. There was a one-minute rest between sets. Jumping sessions ranged from a few minutes in the early stages of the exercise protocol to up to ten minutes by the four month completion (Table 4). All subjects in the jumping group were asked to log their jumping adherence (Appendix C) and their exercise regimen using a calendar that was provided (Appendix D).

Table 4

Jumping Protocol

Jump Training Group			
Jumps x Sets	**Type**	**Jumps x Sets**	**Type**
10 x 3	Two-footed		
10 x 3	Right footed	10 x 3	Left Footed
15 x 3	Two-footed		
15 x 3	Right footed	15 x 3	Left Footed
20 x 3	Two-footed, front to back		
20 x 3	Two-footed, side to side		
25 x 3	Two-footed		
24 x 3	Two-footed, square		
20 x 3	Right Footed	20 x 3	Left Footed
25 x 3	Two-footed		
25 x 3	Two-footed, front to back		
25 x 3	Two-footed, side to side		
24 x 4	Two-footed, square		
25 x 3	Right Footed	25 x 3	Left Footed
25 x 4	Two-footed		
20 x 2	Two-footed, front to back	20 x 2	Two-footed, side to side

Statistical Analysis

All subjects were stratified by oral contraceptive use and matched for OCSI values, then within each pair individually were randomized to the jumping group or control group. Data were tested for normality,

descriptive statistics were computed, and relationships among variables at all time points were examined. Data were transformed using log and inverse transformations to normalize non-normal data. Data were also visually inspected for outliers by osteocalcin standard curve cut points. Those osteocalcin values that were two standard deviations outside the standard curve were excluded from analysis (N=1). Activity logs were assessed to determine total minutes of physical activity by month and total minutes of physical activity over the course of the intervention. Physical activity outside of the jumping protocol was not different between groups, therefore it was not used as a covariate in analyses. For hypothesis 1, repeated measures ANOVA was used to determine differences in bone outcome variables from baseline to month 4 between groups. For hypothesis 2a, Pearson correlations were used to analyze associations between the normalized data at baseline and month 4 in demographic, body composition and bone variables. For hypothesis 2b, repeated measures ANOVA were used to determine between group differences from baseline to months 1, 2, 3, and 4 in bone variables. To determine within group differences utilizing the values at 4 different time points: baseline to months 1, 2, 3, and 4, an univariate analysis was completed with post hoc analysis (Tukey) to determine if differences occurred between the five assessment points in bone variables. All analyses were done using PASW (Version 18).

Results

65 subjects were screened and 32 were deemed eligible to participate in the study. Three of the excluded subjects had thyroid conditions, three had non-normal menstrual cycles, one did not meet the age criteria, one did not schedule baseline testing and the remaining 25 subjects had t-scores above the 1.0 value for their ultrasound measurement. Of the 32 subjects that started the study, four dropped out. One subject became pregnant during the study and therefore dropped out, one subject had an aversion to needles and decided not to continue in the study, one dropped out due to family reasons and another left the state during the study to deal with family issues and was released from the study. Therefore, 28 subjects completed the study. One subject missed one month of blood collection, body composition and ultrasound measurement (month 1). All other subjects completed all five testing days.

Descriptive data are provided in Table 5. There were no significant differences between the jumping and control group at baseline.

Table 5

Baseline Characteristics By Group

Table 5. Baseline Characteristics By Group

	Jumping Group (n=16)		Control Group (n=16)		
	Mean	Standard Error	Mean	Standard Error	P-value
Demographics					
Age (years)	26.44	1.23	26.25	1.25	0.92
Weight (kg)	62.68	3.29	65.85	2.98	0.49
Height (cm)	164.81	1.46	168.75	1.37	0.06
Body Composition					
BMI[a]	23.04	1.06	23.15	1.08	0.95
FFM[b] (kg)	45.37	0.90	46.29	0.88	0.47
Fat Mass (kg)	17.40	2.49	19.56	2.27	0.53
Percent Fat-BIA[c] (%)	26.00	2.33	28.38	2.00	0.44
Percent Fat-DXA[d] (%)	33.59	2.54	33.46	2.04	0.97
Bone Indices					
Right BUA[e] (m/s)	121.77	2.82	124.24	3.33	0.58
Left BUA[e] (m/s)	117.86	3.47	124.63	2.25	0.11
Right SOS[f] (g/cm³)	1592.01	6.88	1581.44	5.77	0.25
Left SOS[f] (g/cm³)	1584.60	6.08	1579.84	4.52	0.54
Right OCSI[g]	106.75	3.18	105.44	3.57	0.79
Left OCSI[g]	102.13	3.12	105.19	2.29	0.44
BMD Total[h] (g/cm²)	1.1446	0.02	1.1726	0.02	0.26
BMC Total[i] (g)	2450.19	59.64	2624.19	70.87	0.07
AP Spine BMD[j] (g/cm²)[j]	1.1829	0.03	1.2383	0.03	0.18
LF BMD Total (g/cm²)[h,k]	1.0507	0.03	1.0450	0.03	0.89
RF BMD Total (g/cm²)[h,l]	1.0488	0.03	1.0448	0.03	0.93

[a]BMI = Body Mass Index (kg/m²); [b]FFM = Fat Free Mass; [c]BIA = Bioelectrial Impedance; [d]DXA = dual energy X-ray absorptiometry; [e]BUA = broadband ultrasound attenuation; [f]SOS = speed of sound; [g]OCSI = os calcis stiffness index; [h]BMD = bone mineral density; [i]BMC = bone mineral content; [j]AP = appendicular; [k]LF = left femur; [l]RF = right femur

Of the remaining 28 subjects, two subjects were dropped after testing for normality due to: 1) extremely high rates of physical activity (training for an Ironman) and 2) serum osteocalcin values that were

extreme outliers as identified by the standard curves and analysis of serum osteocalcin levels. Therefore, 12 subjects in the jumping group and 14 subjects in the control group were included in final data analysis. The compliance for the jumping group to the jumping protocol was 98% (47/48 sessions) for the duration of the study.

Table 6

Comparison Of Between Group Differences In Body Composition, Nutrient Intake and Bone Indices at Baseline and Month 4

Table 6. Comparison of Between Group Differences in Body Composition, Nutrient Intake and Bone Indices at Baseline and Month 4

	Baseline		Month 4		
	J (n=12)	C (n=14)	J (n=12)	C (n=14)	BG[l]
	Mean ± Standard Deviation		Mean ± Standard Deviation		p-value
Body Composition					
Weight (kg)	65.4 ± 13.9	68.7 ± 11.4	64.8 ± 11.8	68.8 ± 11.3	0.33
BMI[a] *(kg/m*[2]*)*	23.9 ± 4.7	23.8 ± 4.2	23.7 ± 4.0	23.8 ± 4.2	0.37
FFM[b] *(kg)*	45.8 ± 3.6	46.9 ± 3.3	45.5 ± 3.2	46.9 ± 3.5	0.27
Fat Mass (kg)	19.6 ± 10.5	21.0 ± 8.8	19.4 ± 9.3	21.0 ± 8.7	0.99
Percent Fat-BIA[c] *(%)*	28.2 ± 9.4	29.8 ± 7.6	28.4 ± 9.6	29.9 ± 7.4	0.81
Nutrition Intake					
Calcium (mg)	513.4 ± 238.6	798.4 ± 606.1	737.7 ± 334.4	968.6 ± 480.4	0.18
Magnesium (mg)	203.7 ± 70.7	276.5 ± 128.9	234.0 ± 91.8	306.2 ± 120.4	0.20
Vitamin K (µg)	84.9 ± 45.9	84.4 ± 69.8	120.3 ± 141.0	83.9 ± 75.7	0.94
Bone Indices					
Right BUA[d] *(m/s)*	121.7 ± 12.2	122.9 ± 13.8	119.0 ± 11.0	123.3 ± 10.2	0.40
Left BUA[d] *(m/s)*	117.6 ± 14.8	123.0 ± 8.5	124.6 ± 10.6	123.7 ± 8.8	0.13
Right SOS[e] *(g/cm*[3]*)*	1592.4 ± 31.8	1581.3 ± 24.6	1580.9 ± 29.2	1579.7 ± 24.5	0.21
Left SOS[e] *(g/cm*[3]*)*	1585.7 ± 27.9	1579.5 ± 19.4	1581.8 ± 20.9	1580.7 ± 20.1	0.40
Right OCSI[f]	106.8 ± 14.2	104.6 ± 15.1	101.8 ± 11.9	104.4 ± 12.8	0.31
Left OCSI[f]	102.3 ± 13.6	104.1 ± 9.3	105.7 ± 10.6	104.9 ± 9.7	0.27
BMD Total[g] *(g/cm*[2]*)*	1.15 ± 0.07	1.18 ± 0.06	1.14 ± 0.06	1.18 ± 0.06	0.37
BMC Total[h] *(g)*	2472.4 ± 245.2	2683.3 ± 228.8	2475.0 ± 235.2	2662.1 ± 227.5	0.36
AP Spine BMD[g,I] *(g/cm*[2]*)*	1.18 ± 0.13	1.25 ± 0.10	1.19 ± 0.12	1.26 ± 0.10	0.36
LF BMD Total[g,j] *(g/cm*[2]*)*	1.04 ± 0.14	1.05 ± 0.11	1.03 ± 0.14	1.05 ± 0.11	0.63
RF BMD Total[g,k] *(g/cm*[2]*)*	1.05 ± 0.14	1.05 ± 0.12	1.04 ± 0.13	1.05 ± 0.11	0.45
Osteocalcin (ng/ml)	9.4 ± 3.5	10.5 ± 6.1	12.8 ± 7.1	12.7 ± 8.0	0.57

[a]BMI = body mass index; [b]FFM = fat free mass; [c]BIA = bioelectrical impedance analysis;
[d]BUA = broadband ultrasound attenuation; [e]SOS = speed of sound; [f]OCSI = os calcis stiffness index;
[g]BMD = bone mineral density; [h]BMC = bone mineral content; [i]AP = appendicular; [j]LF = left femur;
[k]RF = right femur; [l]BG = between groups

* indicates differences between groups from baseline to month 4, $p<0.05$

Hypothesis 1

Repeated measures ANOVA was performed to determine differences in OCSI, BMC, BMD, and serum osteocalcin from baseline to month 4. Table 6 displays the mean bone values (QUS measures, BMD, BMC, and serum osteocalcin) at month 4. There were no significant differences between groups from baseline to month 4 in any bone measures (Table 6). There were no significant between group differences in serum osteocalcin, right and left OCSI, right and left BUA, right and left SOS, total BMD, total BMC, AP Spine BMD, total right BMD, total left BMD, fat free mass, fat mass, weight, BMI, or percent fat based on repeated measures analyses (Table 6). Figures of the change in all bone measures over time from baseline to month 4 are located in Appendix H.

Hypothesis 2a

Pearson correlations to determine relationships between variables were completed at baseline and month 4. Relationships between variables at baseline and at month four are shown in Tables 7 and 8, respectively.

At baseline, significant positive relationships occurred between measures of body composition. Fat free mass was negatively correlated to right and left SOS. However, body composition indices (weight, total percent fat, fat mass, and fat free mass) were positively correlated with total BMC and total BMD. Right and left QUS values were also highly inter-correlated. Right and left OCSI were not significantly correlated

however to total BMC or BMD. There were no significant correlations between any bone values with serum osteocalcin (Table 7).

Table 7

Pearson Correlations At Baseline

Table 7. Pearson Correlations at Baseline

	Age	Weight	Total % Fat	Fat Mass	FFM	BMI	Right SOS	Left SOS	Right OCSI	Left OCSI	Total BMC	Total BMD	Osteocalcin
Age (years)	1	-0.01	0.13	0.05	-0.15	0.05	-0.16	-0.26	-0.02	-0.2	-0.15	-0.15	-0.34
Weight (kg)		1	0.94***	0.99***	0.89***	0.94***	-0.40*	-0.35	-0.26	-0.15	0.53**	0.55**	-0.21
Total % Fat			1	0.97***	0.72***	0.93***	-0.28	-0.22	-0.15	-0.6	0.50**	0.54**	-0.3
Fat Mass (kg)				1	0.79***	0.95***	-0.35	-0.29	-0.23	-0.12	0.53**	0.50**	-0.28
FFM[a] (kg)					1	0.75***	-0.47*	-0.44*	-0.33	-0.21	0.46*	0.60**	0.04
BMI[b] (kg/m^2)						1	-0.29	-0.21	-0.18	-0.09	0.60**	0.39*	-0.34
Right SOS[c] (m/s)							1	0.88***	0.88***	0.61**	0.11	0	0.08
Left SOS[c] (m/s)								1	0.71***	0.72***	0.22	0.04	-0.09
Right OCSI[d]									1	0.79***	0.13	0.16	0.16
Left OCSI[d]										1	0.19	0.26	0.05
Total BMC[e] (g)											1	0.64***	0.3
Total BMD[f] (g/cm^2)												1	-0.01
Osteocalcin (ng/ml)													1

[a]FFM = fat free mass; [b]BMI = body mass index; [c]SOS = speed of sound; [d]OCSI = os calcis stiffness index; [e]BMC = bone mineral content; [f]BMD = bone mineral density

*p < 0.05
**p < 0.01
***p < 0.001

At month 4, as was observed at baseline, body composition variables were significantly correlated. Right and left OCSI were also significantly correlated as were BMC and BMD values (Table 8). As occurred at baseline, weight was negatively correlated to right SOS. AP Spine BMD, total BMC and total BMD were positively correlated to weight and total percent fat. Serum osteocalcin was negatively correlated to age and total percent fat. Serum osteocalcin was not correlated to any other bone values however.

Table 8

Pearson Correlations At Month 4

Table 8. Pearson Correlations at Month 4

	Age	Weight	Total % Fat	Right SOS	Left SOS	Right OCSI	Left OCSI	AP Spine BMD	Total BMC	Total BMD	Osteocalcin
Age (years)	1.00	0.21	0.10	-0.09	-0.04	-0.10	-0.14	-0.29	-0.11	-0.09	-0.45*
Weight (kg)		1.00	0.93***	-0.49*	-0.35	-0.27	-0.19	0.33	0.69***	0.52**	-0.31
Total % Fat			1.00	-0.47*	-0.27	-0.30	-0.07	0.40*	0.62**	0.45*	-0.45*
Right SOS[a] (g/cm^3)				1.00	0.81***	0.85***	0.59**	0.14	-0.12	0.16	-0.10
Left SOS[a] (g/cm^3)					1.00	0.71***	0.8***	0.29	0.00	0.25	-0.12
Right OCSI[b]						1.00	0.71***	0.13	0.05	0.16	-0.03
Left OCSI[b]							1.00	0.19	0.06	0.14	-0.09
AP Spine BMD[c, u] (g/cm^2)								1.00	0.59**	0.67***	-0.29
Total BMC[e] (g)									1.00	0.72***	-0.01
Total BMD[u] (g/cm^2)										1.00	-0.17
Osteocalcin (ng/ml)											1.00

[a]SOS = speed of sound; [b]OCSI = os calcis stiffness index; [c]AP = appendicular; [u]BMD = bone mineral density; [e]BMC = bone mineral content

*p < 0.05
**p < 0.01
***p < 0.001

Hypothesis 2b

A univariate analysis was performed to determine time and group differences and time by group interactions in serum osteocalcin and OCSI. There were significant differences in serum osteocalcin in all subjects from baseline to month 2. No significant overall differences existed for OCSI over time. Also, no between group differences existed. The results indicated that there was no group by time interaction in the change of serum osteocalcin over the 4 assessment points. Figure 8 demonstrates the trend for serum osteocalcin over time in both groups. There were no significant overall differences between groups in right or left OCSI values. There were no significant differences between any bone outcomes between groups or by time (Table 9).

Figure 8

Changes In Osteocalcin Over Time

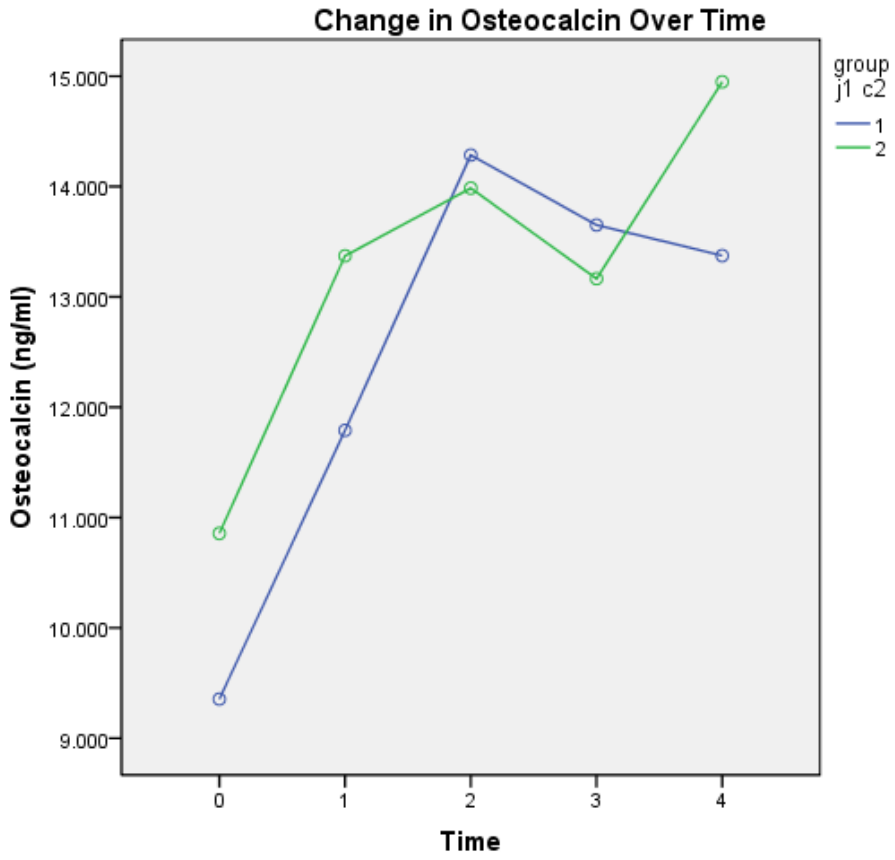


Table 9

Mean Body Composition and Bone Values by Monthly Test

Table 9. Mean Body Composition and Bone Values by Monthly Test

	Baseline		Month 1		Month 2		Month 3		Month 4	
	J (n=12)	C (n=14)	J (n=12)	C (n=13)	J (n=12)	C (n=14)	J (n=12)	C (n=14)	J (n=12)	C (n=14)
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Body Composition										
Weight (kg)	65.40	67.90	65.20	68.80	65.20	67.5	65.4	67.7	64.8	68
	13.90	11.30	13.20	11.50	12.90	11.5	12.4	11.7	11.8	11.3
BMI[a] *(kg/m^2)*	23.90	23.80	23.8	24.20	23.80	23.7	23.8	23.8	23.7	23.8
	4.70	4.20	4.4	4.10	4.40	4.2	4.4	4.4	4	4.2
FFM[b] *(kg)*	45.80	46.90	45.40	46.90	45.30	46	45.7	46.5	45.5	46.9
	3.60	3.30	3.40	3.50	3.50	3	3.1	3.3	3.2	3.5
Fat Mass (kg)	19.60	21.00	19.80	21.80	19.90	21.5	19.7	21.2	19.4	21
	10.50	8.80	10.30	9.00	10.00	9.1	9.9	9.1	9.3	8.7
Percent Fat-BIA[c] *(%)*	28.20	29.80	28.60	30.60	28.80	30.7	28.4	30.1	28.4	29.9
	9.40	7.60	9.80	7.70	9.90	7.6	9.8	7.7	9.6	7.4
Bone Indices										
Right BUA[d] *(m/s)*	121.70	122.90	122.90	120.10	121.80	123.7	119.9	121.5	119	123.3
	12.20	13.80	13.30	12.00	12.30	14.2	10.2	9.5	11	10.2
Left BUA[d] *(m/s)*	117.60	123.00	119.20	124.70	119.20	123	120.7	125.5	124.6	123.7
	14.80	8.50	9.30	10.00	11.40	9.5	12.8	11.5	10.6	8.8
Right SOS[e] *(g/cm^3)*	1592.40	1581.30	1588.60	1575.20	1591.10	1579.4	1585.1	1584.5	1580.9	1579.7
	31.80	24.60	34.50	25.40	34.70	27.1	31.9	22.8	29.2	24.5
Left SOS[e] *(g/cm3)*	1585.70	1579.50	1582.00	1576.90	1589.10	1580	1584.7	1584.5	1581.8	1580.7
	27.90	19.40	26.40	20.80	26.50	22.1	24.9	17.7	20.9	20.1
Right OCSI[f]	106.80	104.60	106.50	101.10	106.50	104.7	103.5	104.5	101.8	104.4
	14.20	15.10	15.90	13.60	15.10	15.9	13.2	12	11.9	12.8
Left OCSI[f]	102.30	104.10	102.30	104.60	104.30	104.2	104	107.3	105.7	104.9
	13.60	9.30	10.90	10.90	11.90	10.9	12.7	11.4	10.6	9.7
Osteocalcin (ug/ml)	9.40	10.50	11.80	13.40	14.30	13.4	12.8	13.1	12.70	14.60
	3.50	6.10	5.60	9.50	9.40	9.2	7.1	9.2	8.00	11.70

[a]BMI = body mass index; [b]FFM = fat free mass; [c]BIA = bioelectrical impedance analysis; [d]BUA = broadband ultrasound attenuation;
[e]SOS = speed of sound; [f]OCSI = os calcis stiffness index
[w] indicates differences from baseline $p < 0.05$

There were no significant differences between groups in QUS measures (right and left SOS, and right and left BUA). There were no time effects within or between groups in SOS or BUA.

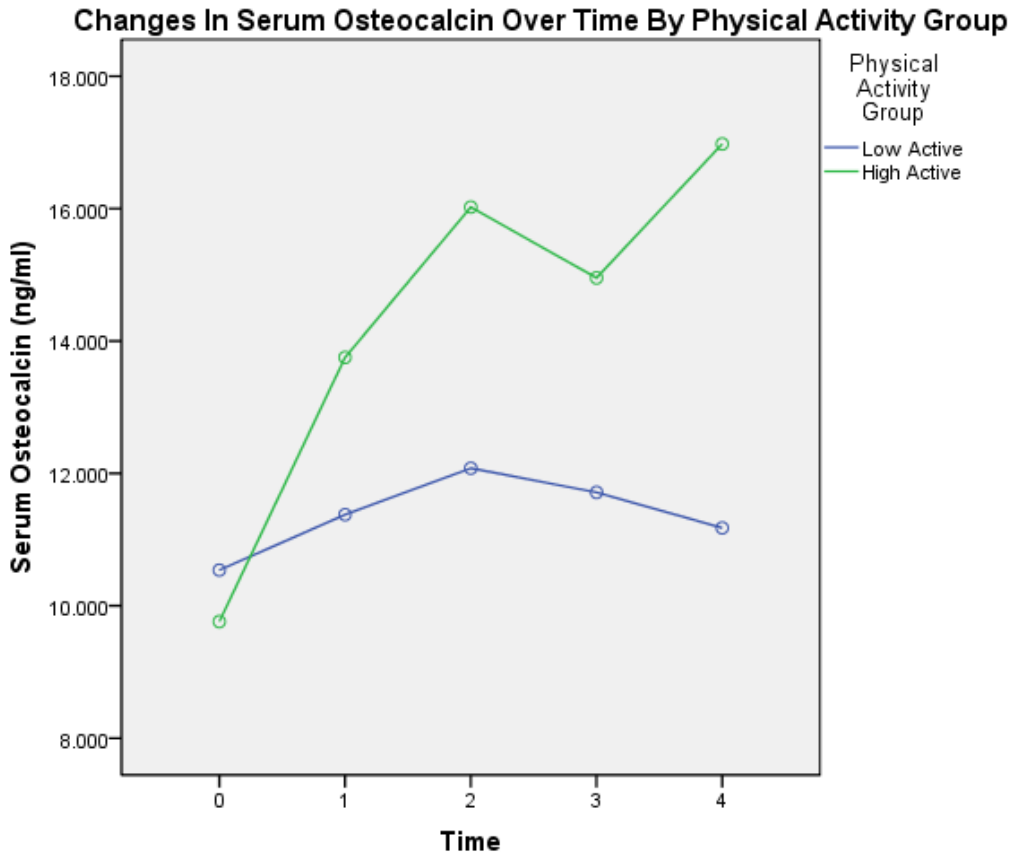
Supplementary Analyses

Reported calcium intake increased significantly in the control group from baseline to month 4 (Table 6). Mean calcium intake did not reach recommended levels (1000 mg/day) in either group. When mean calcium intake was used as a covariate, no changes in bone variables were observed between groups or from baseline to month 4.

This population was moderately active. Subjects had recorded their weekly physical activity time spent outside of the intervention and this was collected each month. Weekly minutes of physical activity was converted to total minutes of physical activity for the 4-month duration. Utilizing the total minutes of physical activity, a median cutpoint was established and a low active verses high active group were created. The mean physical activity levels for the low active and high active group averaged 693.4 ± 443.5 and 3277.3 ± 1975.2 minutes of physical activity, respectively. Serum osteocalcin was significantly higher at months 2 and 4 than at baseline in the high active group ($p < 0.05$) (Figure 9). No significant differences were observed in serum osteocalcin in the low active group. No differences were observed between right and left OCSI between activity groups.

Figure 9

Changes In Serum Osteocalcin Over Time By Physical Activity Group



Discussion

The purpose of this study was to assess the changes in bone status following jump training in young women with low bone strength values as compared to controls and to determine if the blood biomarker of bone formation (serum osteocalcin) was correlated with the quantitative ultrasound (QUS) outcomes and dual energy X-ray absorptiometry (DEXA) measures of bone status. Study participants completed the study with a fairly low attrition rate of 12.5% over the course of the 16-week study.

The average age of participants was about 26 years. The mean of the BMI fell into the normal range for body weight. Percent body fat as indicated by DXA was about 33%. The women in the current study were younger but had similar weight and BMI to the women runners and controls reported by Brahm and colleagues (1997).Adabonyan et al. reported that about 60% of the American population currently meets the physical activity recommendations of at least 150 minutes of physical activity per week (Adabonyan, Loustalot, Kruger, Carlson, Fulton, 2010). On average, less than 50% of the current population met these guidelines, for the 4-month intervention. The average minutes of physical activity for all subjects in the current study over the 4-month intervention was 1,985 minutes. This averages approximately 125 minutes per week.

The average calcium intake for participants at baseline was about 600 mg/day which is well below the average reported calcium intake (932

mg/day) of women in this age range in a US population (Nicklas, O'Neil, & Fulgoni, 2009). Only 6 of 22 subjects completing 24-hour dietary recalls met the recommended calcium intake of 1000 mg/day at baseline or month 4. No subjects met the recommendation for 1000 mg/day at both time points. While this may be alarming, it is estimated that only 30% of the US population is meeting the guidelines for calcium intake (1000 mg/day). Mean magnesium intake was similar in this population as in the US population (Nicklas et al., 2009). Mean Vitamin K intakes for the groups at baseline (84.7 μg/day) did not meet the current recommendations (90 μg/day), however the mean intake at month 4 did meet recommendations (mean 102.1 μg/day) (Panel on Micronutrients, 2001). Only 15.7% of females in the 20-45 year cohort of the NHANES 1999-2004 sample met Vitamin K recommendations. Whereas, 54% of the females in the current study met Vitamin K recommendations during the baseline or month 4 dietary recall completion. Only 14% of participants who completed both the baseline and month 4 recalls were meeting recommendations at both time points which is in line with the NHANES sample. The female mean intake of vitamin K in the NHANES data was 83.8 μg/day whereas the present study ranged from 84.7-102.1 μg/day (Pan, Jackson, 2009). It appears that both calcium and vitamin K recommendations were not being met in this sample and that this is not uncommon in an US population.

Baseline BMD (total, AP spine) and total BMC were lower than that reported by Brahm et al. in endurance trained runners and age-matched controls. However, BUA and SOS values were similar to those reported in the Brahm study (Brahm et al., 1997). Two studies have compared the Achilles Insight ultrasound device with DXA (Jin et al., 2010; Damilakis et al., 2007). Both of these were in older women. The reported OCSI values were 67-87 which was lower than the mean range of 100-107 reported in the current study. Additionally, total BMD values were also approximately 0.2 g/cm^2 lower than the current study (Jin et al., 2010; Damilakis et al., 2007). These lower bone values would be expected since the mean ages for these studies were 50 and 75 years (Jin et al., 2010; Damilakis et al., 2007). Masters et al. indicated that serum osteocalcin values should not be compared across studies due to differences in assay kits (Masters et al., 1994). Therefore, no comparable values of serum osteocalcin levels can be made.

The results indicate that hypothesis 1 should be accepted: "OCSI, serum osteocalcin, BMC, and BMD will not be different between the jumping group and normally active controls from baseline to month 4." There were no significant differences in OCSI, BMC, BMD, or serum osteocalcin between the jumping and control groups at baseline or month four.

The current findings were in contrast to those reported by previous research. McKay et al. found significant differences between groups for

BMC after 4 months in the "Bounce at the Bell" program which consisted of a 10 jump, three-set per day jumping program in children (McKay et al., 2005a). Similarly, Kato et al. observed significant increases in BMD in response to ten maximal jumps per day performed three days per week over six months when compared to controls in a healthy young female population (Kato et al., 2005). Additionally, significant differences in OCSI were observed between a 10-minute and 5-minute jumping group verses controls over a 4-month jumping intervention performed in adolescents (Arnett & Lutz, 2002). Clearly, jump training was determined to be an appropriate and significant stimulus to increase bone indices as measured by DXA and QUS over controls. However, in the current study, jump training did not elicit significant differences between groups for these bone indices.

Hypothesis 2b should be accepted: "there will not be any significant between or within group differences between OCSI and serum osteocalcin at baseline to month 4." There were no significant differences between OCSI and serum osteocalcin between groups at months one, two, and three. However, there were within group differences between OCSI and serum osteocalcin.

Overall, serum osteocalcin values increased over time in both groups. Cross sectional findings by Brahm et al. comparing marathon runners to controls, indicated no differences in serum osteocalcin levels between groups (Brahm et al., 1997). Perhaps serum osteocalcin levels are

not sensitive to exercise training. Or perhaps the curent intervention may not have been of a sufficient intensity to evoke differences between groups. Another explanation may be that the time course to elicit significant serum osteocalcin changes is greater than 4 months. In fact, Christenson (1997) reported that it may take six to nine months to observe changes. In a one-year study of the effects of jumping and/or walking, no differences were observed in osteocalcin from baseline to post-testing following treatment (Shibata, Ohsawa, Watanabe, Miura, & Sato et al., 2003). Thus, it remains unclear if serum osteocalcin is an appropriate index of acute changes in bone formation during an intervention.

Although serum osteocalcin was not different between jumping and control groups when it was compared between activity groups, serum osteocalcin was significantly greater in the high active group than the low active group. Thus, a higher amount of physical activity may result in higher bone formation. Perhaps total physical activity is a stronger stimulus for serum osteocalcin levels than jump training.

No increases were observed in left OCSI or right OCSI values between groups or within groups. Arnett and Lutz (2002) showed increases in OCSI values from baseline to month 4 in their 10-minute verses 5-minute jump roping intervention as compared to controls. Their study was also able to detect differences between their intervention and control group (Arnett & Lutz, 2002). Unfortunately, the current study was not powered to detect changes in OCSI values. While left OCSI values did

increase over time, the variability between people was too great to detect significant increases. Based on the pattern of the changes it likely that the QUS device is sensitive to changes from exercise. However, at least 16 people would be needed per group to determine these differences.

The data indicate that hypothesis 2a is to be accepted: "OCSI, serum osteocalcin, BMC, and BMD will not be significantly correlated to each other at baseline or month 4." There were no significant relationships between OCSI, BMC/BMD, and serum osteocalcin at baseline or month four for both groups overall.

Contrary to previous studies that have evaluated the relationships between the Achilles Insight and DXA measures, Damilakis et al reported significant correlations of 0.69-0.76 between quantitative ultrasound and hip BMD in older women (Damilakis et al., 2007). Lappa and colleagues (2007) reported significantly higher correlations between DXA and QUS in the most elderly subjects when compared to other adult populations (Lappa, Dontas, Trovas, Constantelou, Galanos, & Lyritis, 2007). Jin and colleagues (2010) also reported significant correlations ($r = 0.46$-0.59) between the left OCSI value with BMD at the lumbar spine, total hip, and femoral neck (Jin et al., 2010).

Studies comparing QUS and BMD in children also showed significant relationships ($r = 0.44–0.88$) (Sundberg et al., 1998; Jaworski et al., 1995; Schott et al., 1993). However, these studies utilized a different QUS device than the present study. In summary, these findings suggest

that perhaps DXA and QUS are best correlated when bone values are very low. Or it is possible that relationships between QUS and DXA differ by machines used.

There was no relationship between QUS values and serum osteocalcin. One cross sectional study was found that previously evaluated the relationship of osteocalcin and QUS. McGehee & Johnson (2004), reported significant relationships ($r = 0.90$) between QUS variables and salivary osteocalcin in a middle aged (52-year old) cohort (McGehee & Johnson, 2004). It is not clear why the current study found no significant correlations. It is likely that small sample size and the variability of the sample attenuated the results. This study was only the second study to date that has explored the relationship between osteocalcin and QUS values. Further work in this area is clearly warranted.

Interpretation of the results of this study is complex. The strong correlation between lean mass and BMC is in line with previous findings by others (Daly et al., 2008; Lu et al., 2009). However, body composition variables and QUS were not significantly correlated in this study. In this study, while weight was inversely correlated to SOS, Wunsche et al. found BUA and weight to be correlated (Wunsche et al., 2000). Sundberg and colleagues (1998) also showed a stronger relationship with BUA than SOS to weight. In this study, fat-free mass also negatively correlated to the SOS ($r = -0.39$ - -0.47; $p < 0.05$) at baseline and month 4. It is unclear why this inverse relationship to fat-free mass and weight is occurring. Perhaps,

speed of sound may be measuring other tissues in addition to bone, resulting in a size affect on the SOS measure. Damilakis and colleagues and Cepollaro and colleagues have suggested that not all QUS devices measure SOS equally. They suggest that the Achilles Insight machine used in the present study takes the assessment more quickly than other machines (Damilakis et al., 2007; Cepollaro et al., 2005). The image that the Achilles Insight provides also ensures proper placement of the foot for measurement. This is the only QUS device that provides this image for proper placement. Previous studies utilizing an older device have been reviewed for the possibility that these devices picks up extra "noise" due to the longer duration for assessment and due to the inability to ensure proper placement of the heel in the device (Damilakis et al., 2007; Cepollaro et al., 2005).

This study is not without limitations. No data were collected regarding the serum 25(OH)D values or serum calcium levels in this population. Only subjective measures of dietary intake were utilized pre and post study. According to the ASA24 program, the mean calcium intake of both groups was below the recommended levels for this age group. Therefore, it is possible that the effects of the intervention were negatively influenced by low calcium intakes.

Physical activity levels varied between subjects and were controlled for when possible. Since the study was conducted over the course of two semesters, it appeared that physical activity was high during the breaks

and the beginning of the semester and subsequently dropped off in all subjects in the February and March months, likely due to a busy schedule not allowing for as much leisure time.

It is important to note that the DXA machine system crashed one week after the completion of month 4 testing. It is possible that the unexpected findings were due to latent software defects.

Previous research regarding the effects of exercise on serum osteocalcin is limited. No differences were observed for osteocalcin between groups. Therefore, serum osteocalcin may not be a sensitive measure for assessing acute bone changes over the course of a 4-month intervention when comparing to active controls. The serum osteocalcin values may have been suppressed by the subject's calcium deficiency as indicated by their 24-hour dietary recall results. None of the women in this population met the minimum calcium recommendations (1000 mg/day), thus the bone's ability to respond to a training program was hindered and possibly negated due to this limitation. It is understood that when calcium recommendations are not met, bone formation may be attenuated regardless of the intervention.

Future studies on the effects of exercise training on osteocalcin need to include calcium supplementation to ensure that subjects are meeting recommendations and are therefore able to respond to the training program. Also, subjects should be monitored using accelerometers at baseline to determine normal physical activity patterns

and to ensure similar levels of physical activity at baseline. The results indicate that future interventions may need a larger sample size and/or to be conducted for more than four months to observe measurable changes in BMC, BMD, and QUS.

Conclusion

In summary, there is a relationship between DXA and weight, fat-free mass, and fat mass and an inverse relationship between SOS and weight and fat-free mass, implying that these two measures of bone status are influenced by body size. It is plausible that the SOS measurement may be assessing tissue surrounding the measurement site (the larger the size of the heel, the lower the SOS value), whereas DXA measures (BMC and BMD) increase with body mass. Body composition has been a strong indicator throughout the literature of bone status. Physical activity patterns are a clear determinant of body mass and size and should be taken into consideration as they are also correlated to these bone outcomes. Thus, it is not completely clear why SOS is negatively correlated to weight and fat-free mass while BMD and BMC are positively correlated to these measures.

There were no differences in indices of bone change in response to jump training. No significant correlations existed between the bone variables. Serum osteocalcin does appear to be appropriate for differentiating between those who are physically active as compared to those who are less active. Therefore, for cross-sectional and longitudinal

studies, serum osteocalcin appears to be sensitive to detecting differences between moderate and low amounts of physical activity.

The intention of this study was to attempt to assess whether short-term measurement tools such as serum osteocalcin or QUS are effective for detecting changes in bone over the course of an exercise intervention. This information is important to allow researchers and health professionals to determine an ideal training program for improving bone parameters in at risk women. Calcium supplementation and physical activity monitoring are also essential components for this type of intervention program.

In conclusion, the findings of this study indicate that QUS was not shown to be an effective indicator of bone changes as it relates to bone formation in response to this jump training program. Serum osteocalcin can be used through an intervention to determine increases in physical activity. However, it does not appear sensitive enough to differentiate between changes in physical activity patterns among those who are already active. It is understood that overtraining is a significant risk factor for developing low bone mass in young female athletes. This study may be the first to indicate that approaching and/or meeting the ACSM physical activity guidelines may be influence serum osteocalcin and thus be advantageous for bone health.

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APPENDIX A

IRB INFORMED CONSENT FORM

EFFECTS OF EXERCISE TRAINING ON BONE

CONSENT FORM

Dear Participant:

I am a graduate student under the direction of Dr. Pamela Swan, Associate Professor in the Exercise and Wellness Program in the College of Nursing and Health Innovation (CONHI) at Arizona State University. This study is being conducted through the Healthy Lifestyle Research Center in CONHI. You are being asked to participate in a 4-month research study investigating the effect of jumping on bone changes. As you may know, osteoporosis is a disease of bone fragility usually seen in older women. When one has low bone mass in youth, then they are especially at risk for early onset osteoporosis. There are many accepted exercises protocols for increasing bone mass. However, the optimal program is unknown. Usually, one has to wait many months to see if an exercise program is improving bone strength. A simple ultrasound device may be able to assess incremental (monthly) changes in bone during exercise which can help with designing optimal exercise prescriptions for those with low bone mass.

The purpose of this research study is evaluate the effects of jumping on bone strength, bone density and bone formation in women with low bone mass. The relationship between bone strength (measured by ultrasound of the heel) with bone formation (measured from the blood) will be evaluated monthly to determine if ultrasound would be a good technique for assessing incremental changes in bone mass with exercise.

DESCRIPTION OF RESEARCH STUDY:
Please note if you agree to participate in this study and you meet the eligibility requirements then you will be randomly assigned to one of two groups: a jumping group or a control group. You will not get the option of choosing your group selection. The importance of this study is to identify effects of jumping on bone strength and evaluate the relationship of bone strength to blood measures of bone formation.

Only healthy women between 20-40 years old, who have normal menstrual periods and who have bone strength measures in the low to low-normal ranges (i.e., OCSI scores less than 1.0) will be eligible for the study. You will be excluded from the study if you report that you may be pregnant, have any orthopedic problems that would prevent you from participating or if you plan on moving or leaving the area before the end of the study (May 2011). You will need to answer questionnaires pertaining to your health history, physical activity, and dietary intake.

Once you are deemed eligible for the study and randomized to a group, you will be asked to come to the lab for testing at 5 time periods. Baseline testing and post testing will require about 1.5 hours of time in the laboratory. Month 1, 2 and 3 test periods will require about 20-30 minutes in the laboratory. The jumping group will be provided a monthly jumping protocol that is to be performed at home 3 days per week for the 4 month study duration. Members of the control group will be asked to maintain their normal activities.

ASSESSMENTS:
The following measures will be taken monthly. Weight and percent fat will be measured on a scale called a bioelectrical impedance analysis (BIA) device. BIA test requires you to stand on a scale with metal placements for your feet. These placements send a very weak electrical current through your body to determine your percentage of fat. An ultrasound device will be used to measure each of your heels for bone strength. Your foot will be placed in a box-like device that has inflating balloons around your heel. Rubbing alcohol is sprayed on your foot and the balloons to help ensure a clean ultrasound image. The test takes approximately 2 minutes per foot. A small sample of blood will be drawn from your arm using a small-gauge needle for us to assess biochemical changes in bone formation. This procedure will be done by a Certified Physician's Assistant or phlebotomist.

Bone mineral density will be assessed using DXA (dual energy x-ray absorptiometry) at two times (baseline and at the end of the study month 4). You will be asked to confirm that you are not pregnant

and take a quick urine pregnancy test before you can participate in any test using radiation. You will be asked to take off all jewelry and wear clothing with NO Metal and NO under wire bra. You will be asked to lie on a table for about 15 to 20 minutes while you receive a scan of body low-intensity x-rays. The amount of radiation from the DXA scan is very low and the dosage has been estimated to be equivalent to $1/20^{th}$ of a chest x-ray (similar to the radiation exposure of a long airplane trip).

INTERVENTION PROCEDURES

You will be randomized into one of two groups: jumping (J) or control (C). The exercise training for the jumping group will progressively increase (i.e., the number of repetitions, the number of sets and/or duration) throughout the 4-month training protocol. The jumping group will be asked to jump at home in their bare feet on a hard surface 3 times per week. Jumps will be completed in bare feet so that the shock of the jump is absorbed by the body and not by the shoes. Subjects will be asked to find a safe area to perform jumping free from obstructions that might pose a safety risk. Each month your jumping technique will be evaluated and you will be provided a new monthly jumping routine. Jumping sessions will range from a few minutes in the early stages of the exercise protocol to up to 10 minutes by the 4-month completion. The jumping protocol consists of a progression of jumping repetitions and sets and includes variability in jumping patterns (i.e., one foot, forward back, side to side etc.) to increase the overload across the entire foot surface. There will be a 1-minute rest between sets. Daily jumping sessions can be completed all at once or distributed such that each set is performed at separate times per day (i.e, morning afternoon, evening). All subjects in the jumping group will be asked to log their exercise regimen/adherence using a calendar that will be provided. Subjects will be emailed weekly to check on progress and remind them to complete their logs.

The control group members will be asked to maintain your normal activity routine. You will be asked to come to the lab once per month to monitor bone and body composition changes. You will also be asked to log any physical activity that you performed using a calendar that will be provided. Subjects will be emailed weekly to check on progress and remind them to complete their logs.

Subjects will be taught to complete an online diet recall questionnaire. Subjects will be asked to complete this assessment twice, at the beginning and end of the study.

RISKS:

There are no known risks from using the ultrasound device. It has been used for a number of years on thousands of people safely.

There are possible risks with exercise. We will screen participants based on the American College of Sports Medicine Guidelines, and anyone at high risk for cardiovascular, metabolic or pulmonary disease will be excluded. Anyone with known orthopedic problems will also be excluded. Sore muscles and joint stiffness are probable at the initial stages of the jumping intervention. For safety and to ensure equivalent effects of the jumping intervention all jumping will be done with bare feet on a flat, firm, non-slip surface. The investigator will explain to you the proper form for jumping to reduce the incidence of musculo-skeletal injury during training. Also jumping will be evaluated each month by the investigator to ensure proper technique.

DXA scans are considered the best procedure to measure bone mineral changes, however DXA scans do emit small amounts of radiation. At this point the potential long-term risks from this radiation exposure are uncertain. Please tell us if you have had any radiation exposure in the past year, either from other research studies or from medical tests or care, so we can make sure that you will not receive too much radiation. The amount of radiation that a single DXA scan exposes you to is minimal. It is less than that in dental x-rays (approximately 6 mrem/ scan). This amount is well below the radiation safety guidelines issued by the National Institutes of Health (i.e., 3000 mrem to any tissue in a 13 week period and 5000 mrem in one year). Understand that the effects of radiation from DXA procedures on an unborn child are unknown. Therefore you will be asked to confirm that you are not pregnant and a simple urine pregnancy test will be administered prior to performing any test utilizing radiation. However, be aware, pregnancy

tests are not 100% effective or accurate. If there is a possibility that you are pregnant, you understand that it is your responsibility to inform the investigator and withdraw from participation in the study.

Blood will be drawn (20 ml) from the arm using a small gauge needle by a certified phlebotomist or Physician's Assistant. The needle prick can be painful and bruising is possible. Nausea and fainting is also possible when drawing blood.

BENEFITS:
There may be no direct benefit to you, the possible benefit of your participation in this study may be increased bone mass and reduced risk for osteoporosis. You may also reduce body fat. The indirect benefit is that we will be able to determine if the ultrasound device is able to assess incremental changes in bone during exercise, which can help us design optimal exercise prescriptions for people with low bone mass.

NEW INFORMATION:
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIAL:
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. In order to maintain confidentiality of your records, Pamela Swan and Kristin Heumann will keep the files locked in a cabinet. Each participant will be given a code for testing procedures that will not be attached to any of the documents that include your name. Only Dr. Pamela Swan and Kristin Heumann will have access to these files.

WITHDRAWAL PRIVILEGE:
Your participation in this study is voluntary. If you choose not to participate or to withdraw from the study at any time, there will be no penalty. The results of the research study may be published, but your name will not be used.

If applicable: Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

COSTS AND PAYMENTS:
The only costs are related to time and travel to and from the facility. Parking will be provided for you while you are performing testing or exercise sessions if you do not have an ASU Parking Pass.

COMPENSATION FOR ILLNESS AND INJURY:
If you agree to participate in the study, then your consent does not waive any of your rights. In the event of an emergency, '911' will be called and emergency technicians will be brought to the ISTB building to assist you. However no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT:
If you have any questions concerning the research study or your participation in this study before or after consent, please call Kristin Heumann at (480) 206-8637 or Dr. Swan at (480) 727-1934. If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965-6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may

choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be offered to you.

Your signature below indicates that you consent to participate in the above study.

Subject's Signature	Printed Name	Date

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided offered the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

ASU IRB Approved
Sign AS for Carol Johnston
Date 10/7/10 – 9/7/11

Initials _____

APPENDIX B

PROCEDURES FOR OSTEOCALCIN

Biomedical Technologies Inc.

378 Page Street • Stoughton, MA 02072 USA • Phone: (781) 344-9942 Fax: (781) 341-1451 **Web:** www.btiinc.com

Procedural Change:

Please refer to page 2, #4, and #5. Two antisera are now used. Both are provided as concentrates. There is an additional pipetting and incubation step. Please read the manual carefully before starting.

Biomedical Technologies Inc.
378 Page Street • Stoughton, MA 02072 • USA • Phone: (781) 344-9942 Fax: (781) 341-1451 **Web**: www.btiinc.com

INTACT HUMAN OSTEOCALCIN EIA KIT
Catalog No: BT- 460

96 Well Tests Storage 4°C

For the Measurement of Human, Monkey, Dog and Bovine Osteocalcin
in Serum or Heparinized Plasma.

Introduction

Osteocalcin, the vitamin K-dependent protein of bone, is a specific product of the osteoblast. It is distinguished by its small size (5800 daltons) and the presence of gamma-carboxy-glutamic acid (Gla). In the presence of ionic calcium, the Gla residues allow a specific conformational change in the protein which in turn promotes osteocalcin binding to bone mineral and subsequent accumulation in bone matrix. While osteocalcin is primarily deposited into the extracellular matrix of bone, a small amount can be detected in the blood. Circulating osteocalcin is thought to reflect that portion of newly synthesized protein that does not bind to bone but is released directly into the circulation.

Several recent studies suggest that there are various forms of osteocalcin in the circulation and that different antibodies detect different subforms or fragments of osteocalcin. Many polyclonal antibodies detect both intact and fragmented osteocalcin. The physiological significance of such osteocalcin fragments is unclear but they may be derived from osteoclastic resorption of matrix, osteoblastic synthesis, systemic catabolism, or all of these.

Principal of the Assay

The assay measures only intact osteocalcin, which is synthesized de novo by the osteoblast, and it eliminates any potential confounding interference by circulating fragments. The assay is a sandwich EIA which utilizes monoclonal antibodies directed toward the amino- and carboxy- terminal regions of the protein. It recognizes only intact osteocalcin, requiring the full 49 residue protein for detection. It is rapid, sensitive and reliable.

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FOR RESEARCH USE ONLY **(REV. DATE: 11/10)**

Reagents: Description and Preparation
All reagents stable at 4°C for 6 months.

1. Sample Buffer. **BT-471**. One 60 ml bottle. Store at 4°C. Stable for 6 months.

2. Phosphate-Saline buffer concentrate (Wash buffer). **BT-492** One 100ml bottle. Dilute contents to 500 ml with deionized water. Store at 4°C. Stable for 6 months.

3. Osteocalcin Standard. **BT-463**. Five vials, Lyophilized. Reconstitute each vial with 0.5ml deionized water (use 0.50ml volumetric pipet), replace stoppers and let stand for 5 minutes. Mix each vial end-over-end several times to obtain a clear solution. Store these reconstituted standards frozen at -20°C (Stable for 6 months). Thaw completely and allow reconstituted standards to reach room temperature prior to use. Stable for 2 freeze thaw cycles.

4. Biotinylated Antiserum. **BT-464**. One Vial, 0.25ml. Biotinylated antibody to human osteocalcin. Dilute with sample buffer sufficient antiserum for current use and according to dilution ratio printed on the label. Store at 4° C. Stable for 6 months.

5. Native Human Osteocalcin Antiserum. **BT-466**. One Vial, 0.5ml. Dilute contents with 10ml of sample buffer and use immediately. This is a 1:20 dilution. Dilute only enough antibody for current use. Store at 4° C. Stable for 6 months.

6. Streptavidin Horseradish Peroxidase. **BT-475**. One Vial, 11ml. Store at 4°C. Stable for 6 months.

7. Peroxidase Substrate. TMB (3,3',5,5' Tetramethylbenzidine). **BT-497**. One Vial. Store at 4°C. Stable for 6 months.

8. Stop Solution (1M HCl +1M H3PO4). **BT-499**. One Vial, 12ml. Store at 4°C. Stable for 6 months.

9. Hydrogen Peroxide Solution. **BT-498**. One Vial. Store at 4°C. Stable for 6 months.

10. Human Osteocalcin Controls. Two Vials. Add 200ul deionized water to each, let stand 10 minutes at room temperature, gently mix by inversion. (**BT-469H**, High control 25ng/ml) and (**BT-469L**, Low control 5ng/ml).

11. One 96-Well (8 strip removable well) plate, coated with 1-19 monoclonal antibody.

Other Supplies Required
1. ELISA Plate Reader which can measure absorbance at 450nm. 2. Pipettes: 100ul and 1-25ul micropipettes. 3. A plate washer is recommended for washing. 4. Deionized water.
Precautions
Some components of this kit contain isothiazolones (5ppm) as preservative. Stop solution contains sulfuric acid. Keep these materials away from the skin and eyes.

Sample Collection and Storage

All samples (serum, plasma, cell culture media, etc.) should be aliquoted and stored at -20°C. For long term storage (>1month) store at -70°C. All samples should undergo only one or two freeze-thaw cycles. Serum or Heparinized plasma are ideal for blood samples. Dog serum can be run neat or diluted 1:1. Use the diluent buffer (**BT-471**) for any sample dilutions. Since bovine osteocalcin (present in bovine serum) is virtually identical with human osteocalcin (and reacts in this assay) it is necessary to wash cells with/and grow in serum free media 24-48 hours prior to taking samples.

Assay Procedure

All reagents should be at room temperature.

1. Please refer to page 2 for preparation of reagents. All reagents **must be at room temperature.**

2. Remove microtiter plate from resealable bag. Strips not used immediately should be removed from the frame and resealed in the bag for future use.

3. Add 25ul diluent buffer (zero or blank), standards, samples and controls to appropriate wells followed by 50ul **Native** Osteocalcin antiserum (**BT-466**) The entire plate should be completed in 15 minutes or less. Gently swirl about 1 minute. Cover tightly and incubate at room temperature, 1hour.

4. Add 50ul of diluted **Biotinylated** antiserum (**BT-464**) to all wells. Swirl as above, incubate at room temperature for 1 hour.

5. Aspirate completely and wash the plate 3 times with 0.3ml phosphate-saline wash buffer. Add 100ul Streptavidin-Horseradish Peroxidase reagent to all wells. Swirl and then incubate at room temperature for 30 minutes.

6. Mix one volume of TMB solution (**BT-497**) with one volume of Hydrogen Peroxide solution (**BT-498**) and put aside (only mix an amount sufficient for the number of wells in use). Wash plate as in step 4. immediately add 100ul of substrate mix to all wells, incubate at room temperature, in the dark, 10 minutes.

7. Add 100ul stop solution to all wells, swirl, measure absorbance immediately at 450nm. Collect data.

Notes

1. Add stop solution in the same order to the plate as the substrate.
2. Before absorbance measurements are taken, be sure there are no air bubbles floating on top, and the bottom of the wells are clean and dry.
3. Avoid crosscontamination by using new pipet tips for each standard and sample. Dispense samples and standards at bottom of the wells and reagents near the top. Do not agitate or strike the plate so briskly as to cause droplets of liquid to fly up from the wells.

Calculation of Results

Average duplicates for all determinations. Subtract the zero (blank) standard from all averaged readings. Plot net optical density of the standards vs. log of the concentration of each, draw the best curve. Obtain concentration of each unknown from this standard curve. Always generate a standard curve for each new assay.

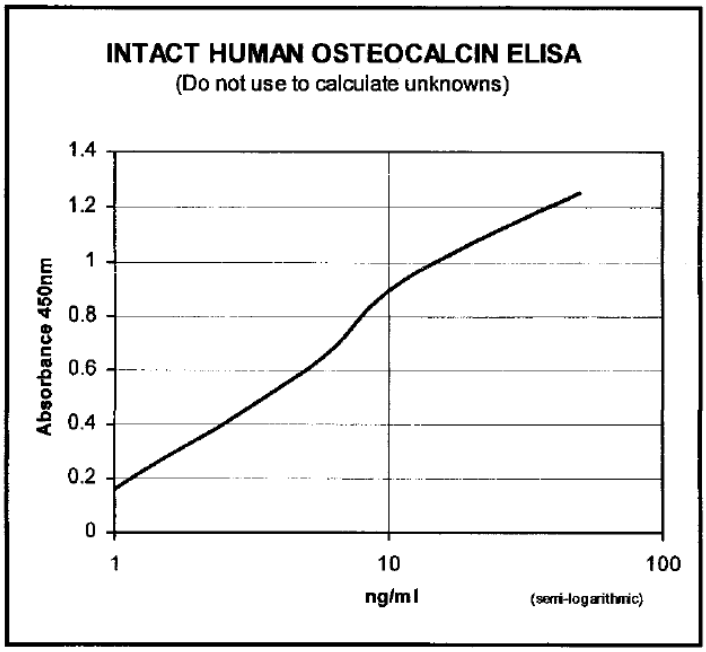
Specifications

Sample size: 25ul
Assay time: 3 ½ hours
Sensitivity: 0.5 ng/ml
Working range: 1.0-50ng/ml (450nm)
Intraassay variation: 7% (95% limits)
Interassay variation: 10.5% (95% limits)
Reference Interval for normal adult males and premenopausal females:2-7ng/ml.
High Dose "Hook" at >250ng/ml.

Typical Data
(Do not use for Determination of Unknowns)

Standard (ng/ml)	A_{450nm}	Avg-blank
0.00 (Blank)	0.149	
0.00	0.146	0.147
1.00	0.294	
1.00	0.320	0.160
5.00	0.745	
5.00	0.850	0.605
10.00	1.051	
10.00	1.031	0.894
25.00	1.242	
25.00	1.286	1.117
50.00	1.409	
50.00	1.393	1.254

Typical Standard Curve



APPENDIX C

JUMPING DIRECTIONS AND LOG

Jumping Directions

Jumping should be performed in bare feet on a hard surface (tile, concrete, wood). Please do NOT jump on carpet or mats.

The jumping days are designed to be at LEAST 24 hours apart.

Ideally, you should train every other day

- Monday, Wednesday, Friday
- OR
- Sunday, Tuesday, Thursday
- OR
- Tuesday, Thursday, Saturday

The jumping should be performed on the balls of your feet; your heels should not touch the ground in between jumps.

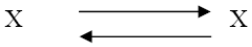
Two-footed jumps are performed by jumping and landing on two feet simultaneously.

Right-footed jumps are performed jumping only on the right foot.

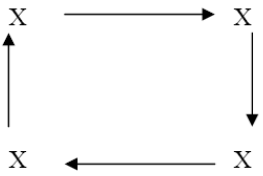
Left-footed jumps are performed jumping only on the left foot.

Front to back jumps are performed by jumping (with two feet) in front of you and back to the starting position (like a bell goes back and forth).

Side to side jumps are performed by jumping (with two feet) from where you start moving to the right and back to the starting position (like a skier on the mountain).



Square jumps are performed by jumping (with two feet) from your starting position, jumping forward, to the right, backward and to the left. Upon completion of one square you should be back to the position where you started. Each jump counts as a jump (4 jumps per square).



Jumping Group		
Week 1, Day 1		
	Type	Completed - Mark with X
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	

Jumping Group		
Week 1, Day 2		
	Type	Completed - Mark with X
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	

Jumping Group		
Week 1, Day 3		
	Type	Completed - Mark with X
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	
1 minute Rest		
10 jumps	2 footed	

Jumping Group		
Week 2, Day 1		
	Type	Completed - Mark with X
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 2, Day 2		
	Type	Completed - Mark with X
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 2, Day 3		
	Type	Completed - Mark with X
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		
10 jumps	Right footed	
10 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 3, Day 1		
	Type	Completed - Mark with X
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	

Jumping Group		
Week 3, Day 2		
	Type	Completed - Mark with X
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	

Jumping Group		
Week 3, Day 3		
	Type	Completed - Mark with X
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	
1 minute Rest		
15 jumps	2 footed	

Jumping Group		
Week 4, Day 1		
	Type	Completed - Mark with X
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
Jumping Group		
Week 4, Day 2		
	Type	Completed - Mark with X
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
Jumping Group		
Week 4, Day 3		
	Type	Completed - Mark with X
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		
15 jumps	Right footed	
15 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 5, Day 1		
	Type	Completed - Mark with X
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	

Jumping Group		
Week 5, Day 2		
	Type	Completed - Mark with X
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	

Jumping Group		
Week 5, Day 3		
		Completed - Mark with X
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	
1 minute Rest		
20 jumps	Front to back - 2 footed	

Jumping Group		
Week 6, Day 1		
	Type	Completed - Mark with X
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

Jumping Group		
Week 6, Day 2		
	Type	Completed - Mark with X
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

Jumping Group		
Week 6, Day 3		
	Type	Completed - Mark with X
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

Jumping Group		
Week 7, Day 1		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 7, Day 2		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 7, Day 3		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 8, Day 1		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 8, Day 2		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 8, Day 3		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 9, Day 1		
	Type	Completed - Mark with X
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
Jumping Group		
Week 9, Day 2		
	Type	Completed - Mark with X
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
Jumping Group		
Week 9, Day 3		
	Type	Completed - Mark with X
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		
20 jumps	Right footed	
20 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 10, Day 1		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 10, Day 2		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 10, Day 3		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 11, Day 1		
	Type	Completed - Mark with X
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	

Jumping Group		
Week 11, Day 2		
	Type	Completed - Mark with X
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	

Jumping Group		
Week 11, Day 3		
		Completed - Mark with X
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	
1 minute Rest		
25 jumps	Front to back - 2 footed	

Jumping Group		
Week 12, Day 1		
	Type	Completed - Mark with X
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	

Jumping Group		
Week 12, Day 2		
	Type	Completed - Mark with X
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	

Jumping Group		
Week 12, Day 3		
	Type	Completed - Mark with X
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	
1 minute Rest		
25 jumps	Side to side, 2 footed	

Jumping Group		
Week 13, Day 1		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 13, Day 2		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 13, Day 3		
	Type	Completed - Mark with X
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	
1 minute Rest		
24 jumps	Square, 2 footed	

Jumping Group		
Week 14, Day 1		
	Type	Completed - Mark with X
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 14, Day 2		
	Type	Completed - Mark with X
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 14, Day 3		
	Type	Completed - Mark with X
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		
25 jumps	Right footed	
25 jumps	Left footed	
1 minute Rest		

Jumping Group		
Week 15, Day 1		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 15, Day 2		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 15, Day 3		
	Type	Completed - Mark with X
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	
1 minute Rest		
25 jumps	2 footed	

Jumping Group		
Week 16, Day 1		
	Type	Completed - Mark with X
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

Jumping Group		
Week 16, Day 2		
	Type	Completed - Mark with X
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

Jumping Group		
Week 16, Day 3		
	Type	Completed - Mark with X
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	
1 minute Rest		
20 jumps	Front to back, 2 footed	
1 minute Rest		
20 jumps	Side to side, 2 footed	

APPENDIX D

PHYSICAL ACTIVITY LOG

Week _____						
Activity Log						
Sunday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Monday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Tuesday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Wednesday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Thursday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Friday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration
Saturday	Activity	Duration	Activity	Duration	Activity	Duration
	Activity	Duration	Activity	Duration	Activity	Duration

APPENDIX E

HEALTH HISTORY QUESTIONNAIRE

Participant ID:_____

Demographic, Physical Activity and Health Questionnaire

1. **Date of Birth: _____**

2. **Are you of Hispanic, Latino or Spanish origin?**
   No, not of Hispanic, Latino or Spanish origin 1
   Yes, Mexican, Mexican-American or Chicano 2
   Yes, Puerto Rican 3
   Yes, Cuban 4
   Yes, another Hispanic, Latino, or Spanish origin (please specify):
   _____

3. **Which one or more of the following would you say is your race or ethnicity?**
   White 1
   African American or Black 2
   American Indian or Alaskan Native 3
   Asian, Native Hawaiian or other Pacific Islander 4
   Other (please specify):
   _____

4. **Have you ever been on a weight reducing or special diet program?**

   _____No, never.

   _____Not now but I was in the past.

   _____ # of times in the past.

   List the kind of diet program._____

   _____ Yes, I am currently on a diet.

   I am currently eating about _____ calories a day.

   How long have you been on this diet ? _____ days.

   How many times have been on this type of diet in the past?_____

4. **Do you consume caffeinated (Coke, Coffee type) beverages? _____Yes _____No**

   If yes, how many cans/cups per day? _____

   If yes, how many cans/cups per week? _____

5. **Have you ever broken a bone?**
   If so, please list all bones and number of times they have been broken:
   _____

   _____

6. **How would you rate your lifestyle?** **_____Not active** **_____Active**
   _____Somewhat active **_____Very active**

7. **Please list any sports or other physical activities you have participated in regularly. Please tick the boxes to indicate how old you were for each sport/activity and how many years you participated for.**

Age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Activities															
Activities	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

8. **Please circle the total practice or exercise time you spend <u>in each category</u> for an average week.**

Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the <u>last 12 months</u> and indicate the average frequency (sessions per week):

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

List any / all sports that you have participated, <u>in the past</u>:
Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Sport:_____
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

List any/ all physical activity (weight lifting, jogging, hiking) you participated in but were not on a competitive team:

Activity:_____
Hours per week: 0 2 3 4 5 6 7 8 9 10+

Activity:_____
Hours per week: 0 2 3 4 5 6 7 8 9 10+

Activity:_____
Hours per week: 0 2 3 4 5 6 7 8 9 10+

9. Please answer (Yes/No) if you have or have <u>ever</u> been diagnosed with any of the following diseases or symptoms:

Check YES or NO next to EACH condition listed.

	YES	NO		YES	NO
Heart attack			Chest Pain		
Hyperparathyroidism			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Renal Disorder			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorders			Emotional Problems		

10. Please list any other diseases or disorders that are not listed above that you have been diagnosed with:

_____

11. Please list ALL medications that you are currently taking or have taken in the last month.

_____

_____

12. Please list any supplements/vitamins that you are currently taking:

_____

13. Have any of your relatives been diagnosed with osteoporosis? Please Circle.

Mother Father Aunt Uncle Grandmother Grandfather

14. Have you started your menstrual cycle? _____Yes _____ No

If YES—how often do you menstruate? (every 28 days, every 3 months, etc.)

_____

When did your last menstrual period begin? _____

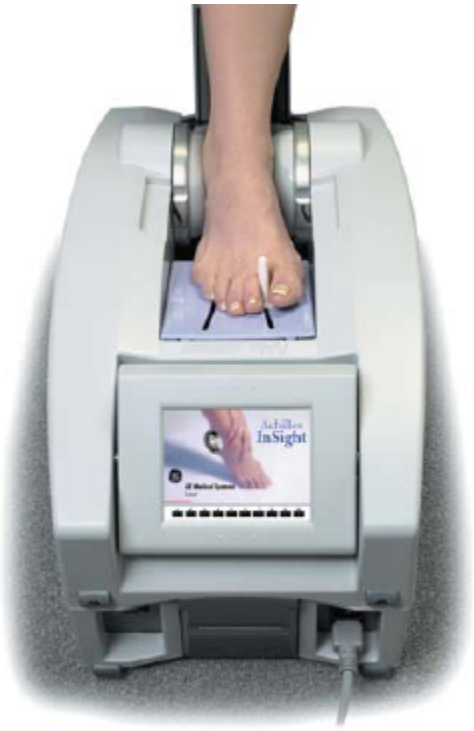
15. Are you currently taking hormone supplements (birth control pills)? ___Yes ___ No

16. Have you ever taken hormone supplements in the past? ___Yes ___ No

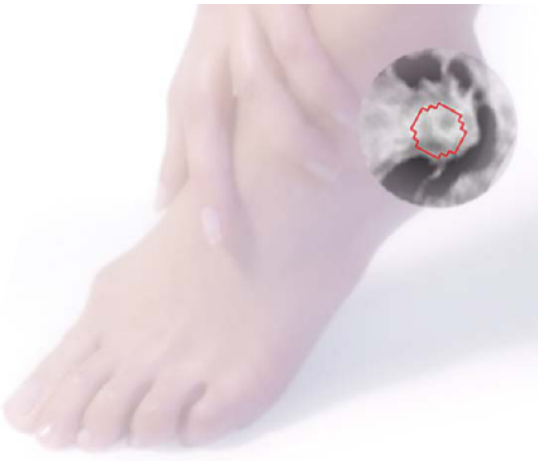
APPENDIX F

QUANTITATIVE ULTRASOUND IMAGE

Achilles Insight Device



Point of Measurement



APPENDIX G

24-HOUR DIETARY RECALL INSTRUCTIONS

Hi _____,

Here are the instructions for completing the 24-hour dietary recall. If, at any point you have questions please call me or text: 480-206-8637.

First, click on the following link: https://asa24beta.westat.com
You will have to disable all pop-ups from your computer.

Once there, use your
Username: _____
Password: _____

Press continue to watch the tutorial. This will explain how to complete your recall. Sound is very helpful throughout the food recall so it is important that you can hear the recommendations made to you at each step.

After you complete the tutorial you will be taken to a welcome screen. Press the green check box to continue. Select the date for yesterday (if it is not selected). Please make sure to only complete your food recall for yesterday.

Use the drop down boxes to select your meals, time, and location. Once you have created a meal you can select your foods. When you are done selecting the foods that you have selected for that meal, click add a meal to add your meals. If you need to change something you can use the delete or edit a meal tabs also. When you have completed all of your meals for the full day yesterday click "done with all meals."

On this page, select the amount of food you consumed for each item.
When you have completed the recall click "done with all meals." The program will take you through some extra questions to verify that everything is correct and then if it is click the green check mark button.

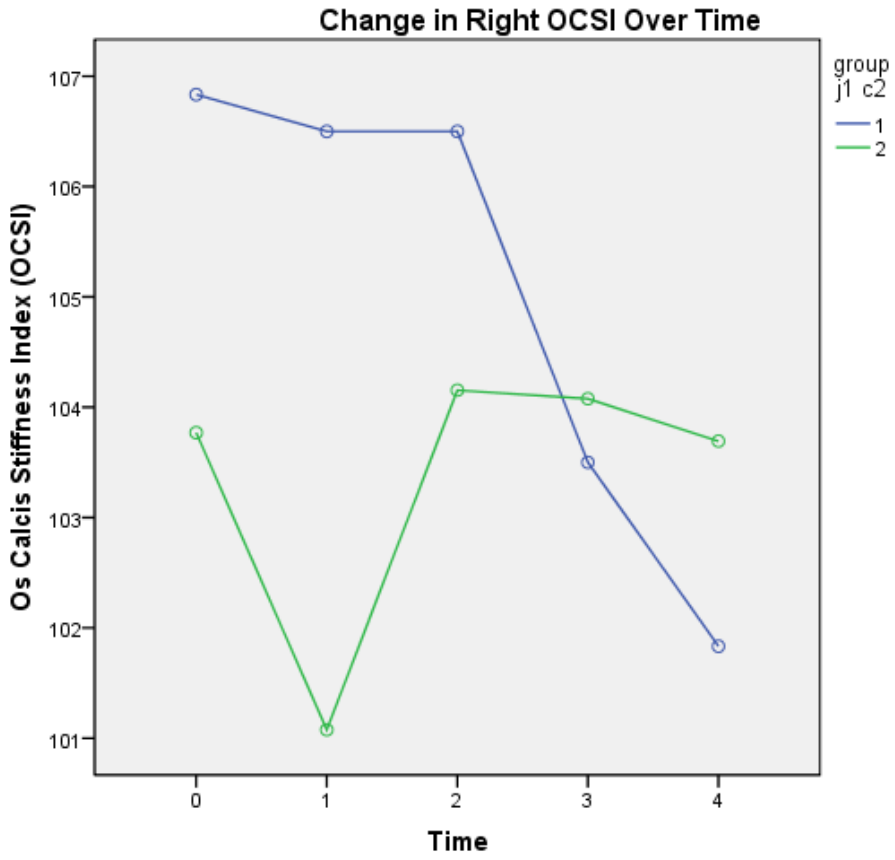
When you are complete you will receive a message thanking you for completing the ASA 24 program.

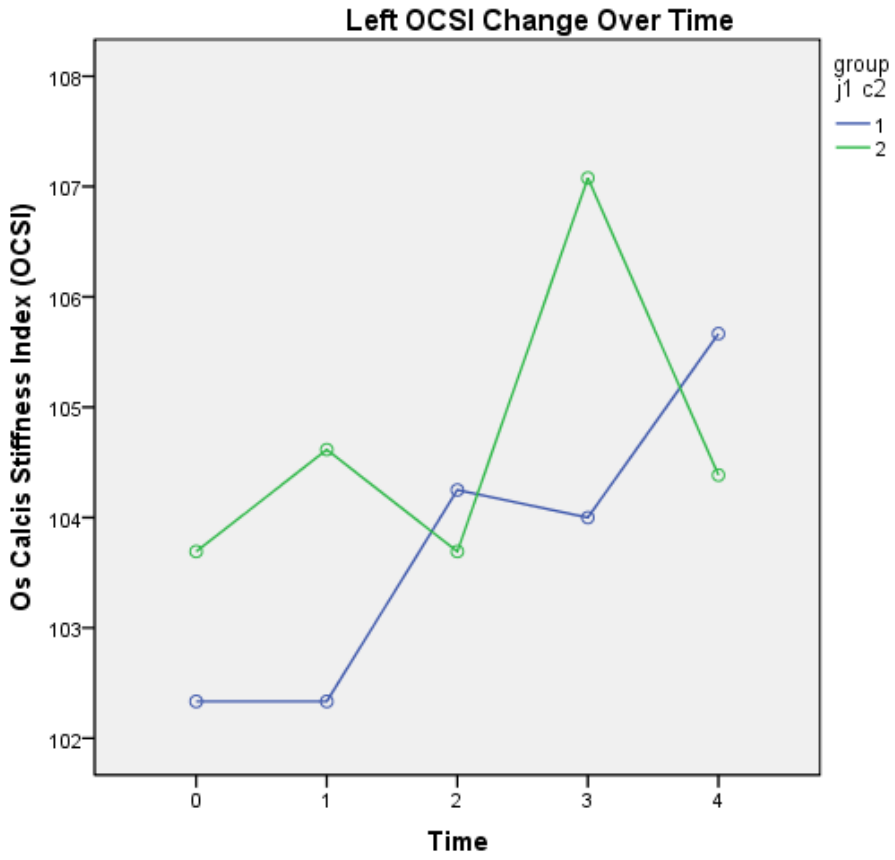
Again, please let me know if you have any questions regarding how to use the program.

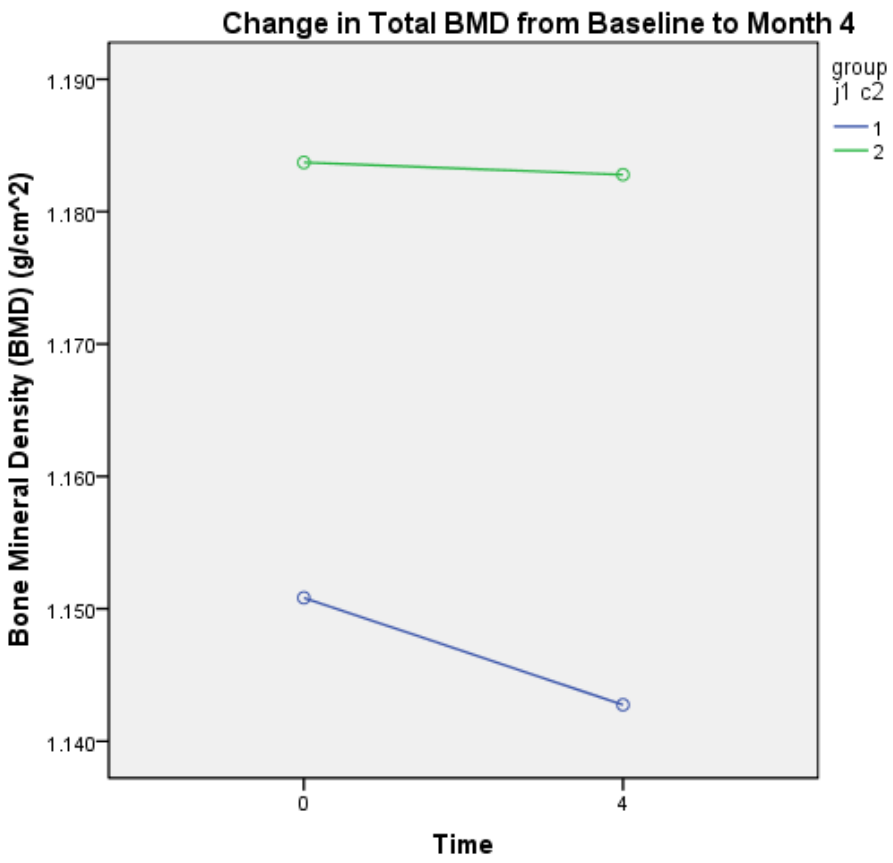
Thanks,

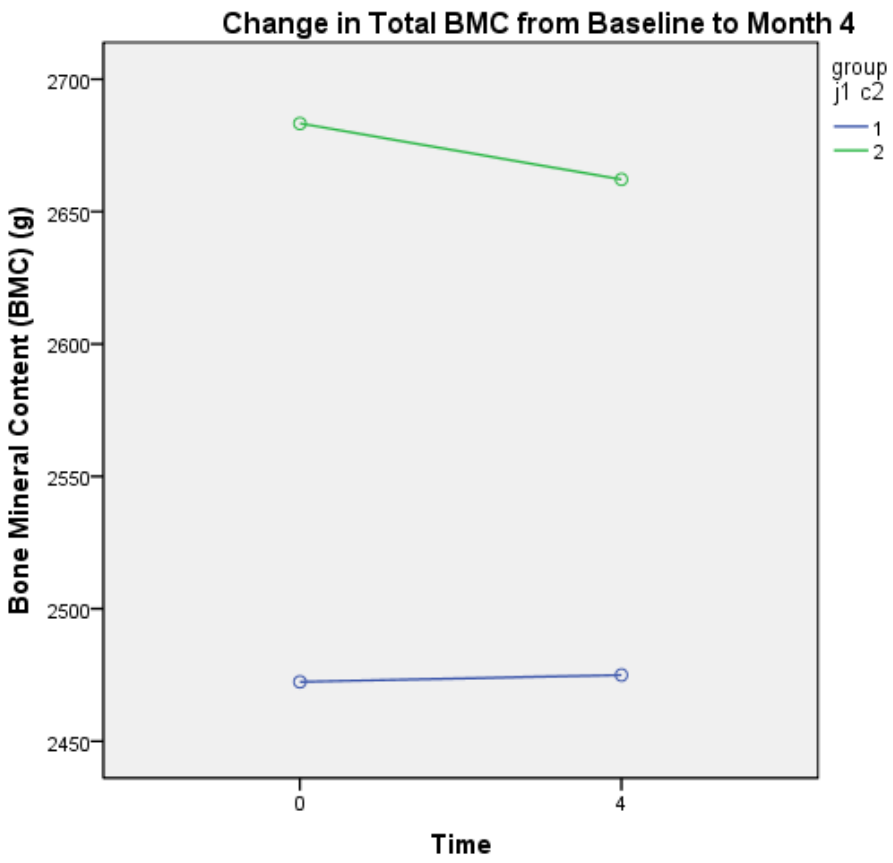
APPENDIX H

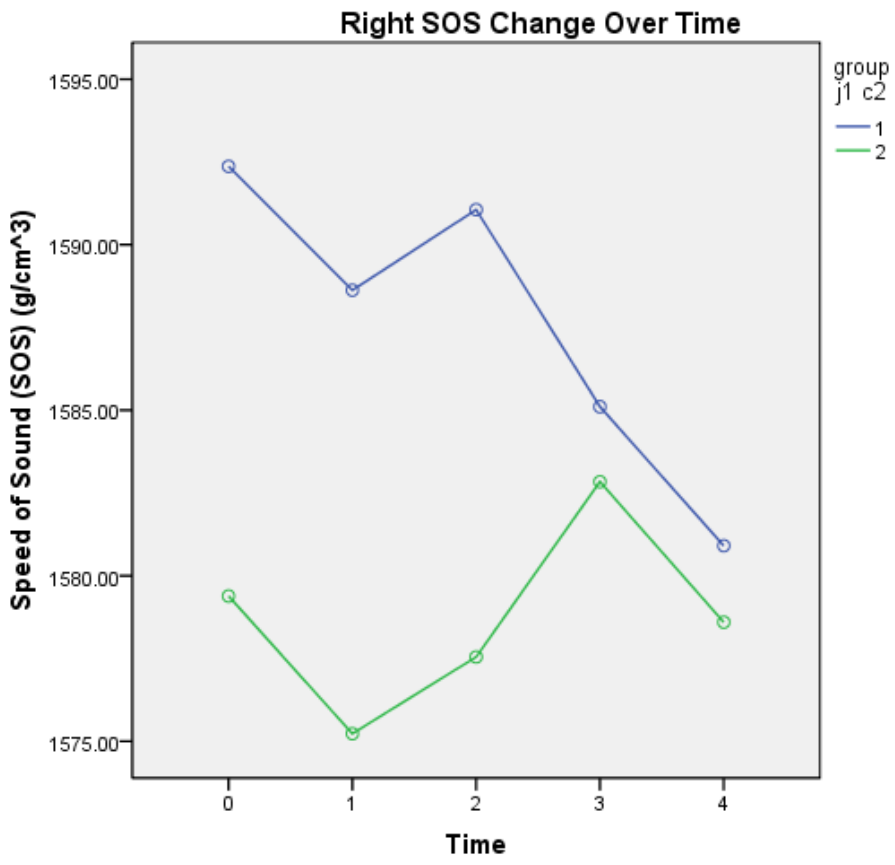
BONE FIGURES OVER TIME

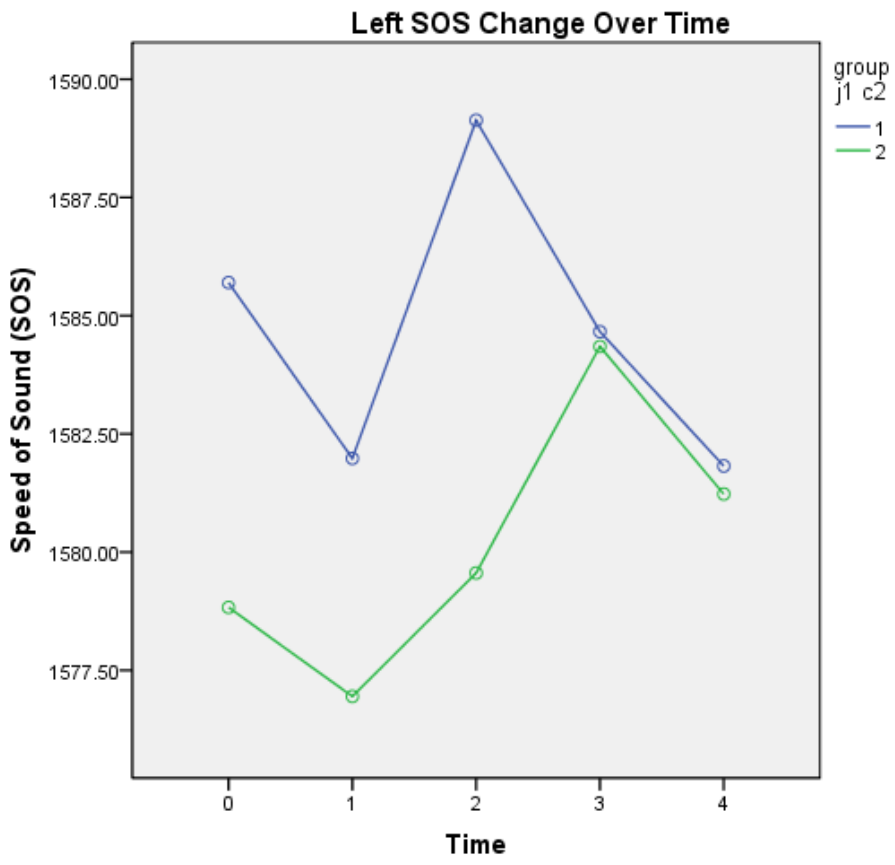

Change in Right OCSI Over Time
Os Calcis Stiffness Index (OCSI)
107
106
105
104
103
102
101
0
1
2
3
4
Time
group
j1 c2
1
2

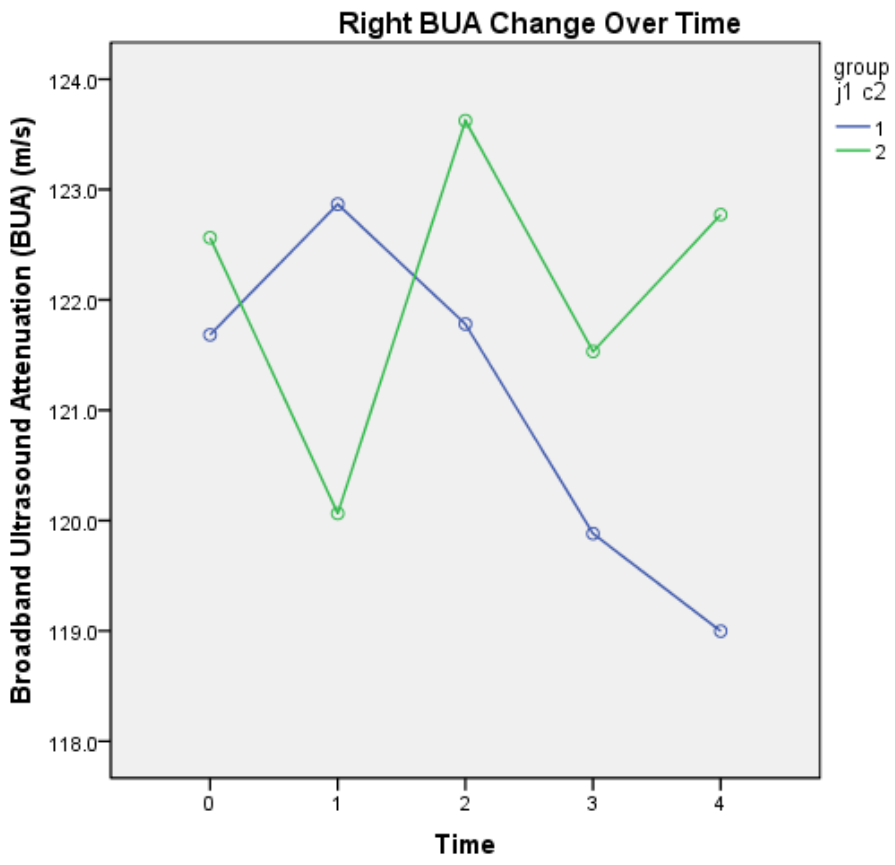

Left OCSI Change Over Time
Os Calcis Stiffness Index (OCSI)
108
107
106
105
104
103
102
0
1
2
3
4
Time
group
j1 c2
1
2

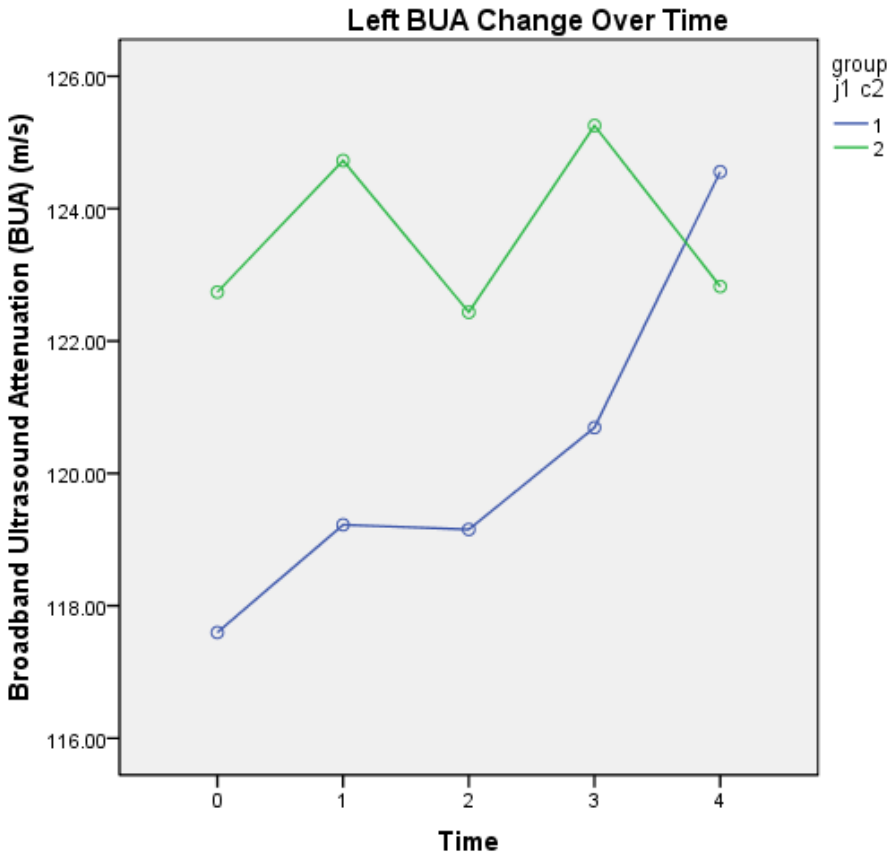

Change in Total BMD from Baseline to Month 4
Bone Mineral Density (BMD) (g/cm^2)
1.190
1.180
1.170
1.160
1.150
1.140
0
4
Time
group
j1 c2
1
2

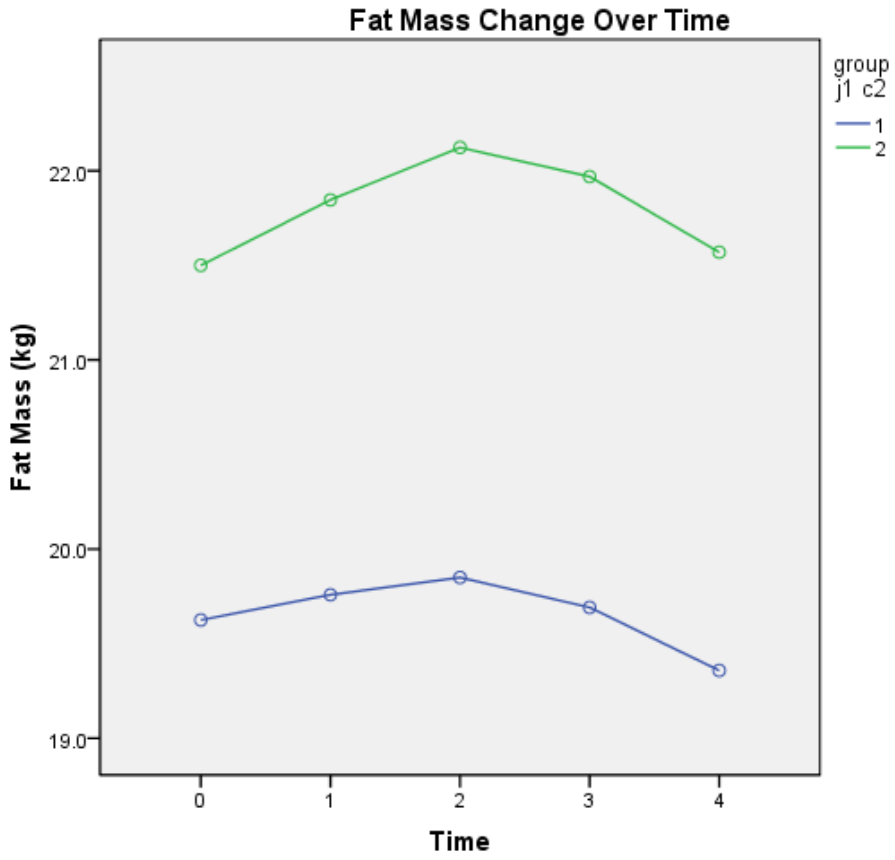

Change in Total BMC from Baseline to Month 4
Bone Mineral Content (BMC) (g)
2700
2650
2600
2550
2500
2450
0
4
Time
group
j1 c2
1
2

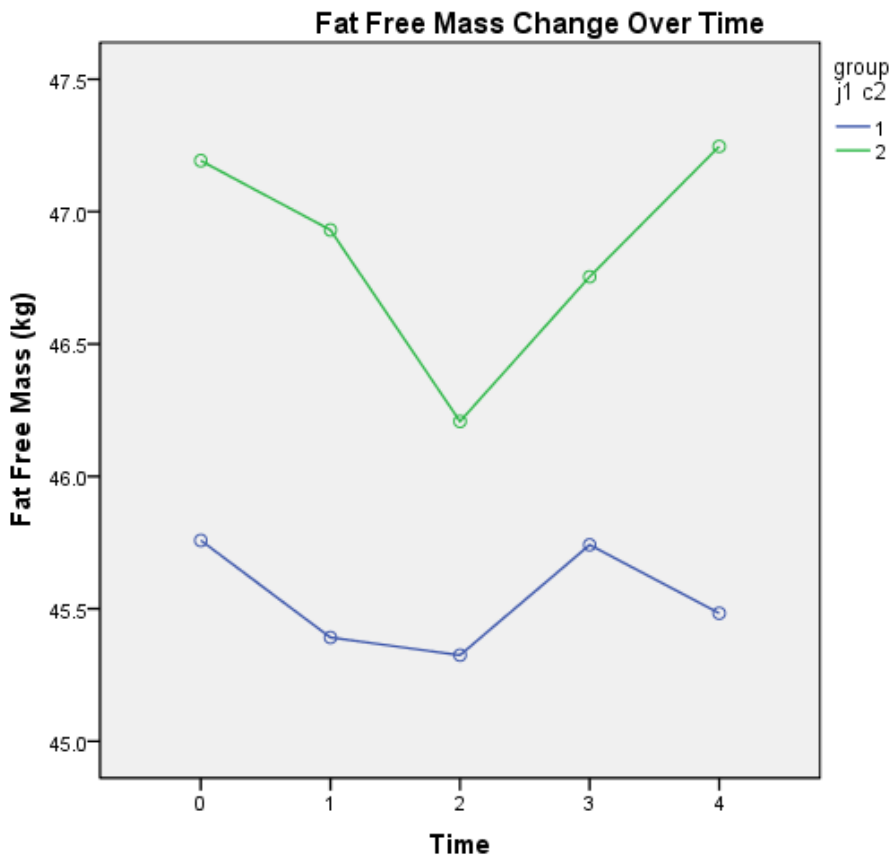

Right SOS Change Over Time
Speed of Sound (SOS) (g/cm^3)
1595.00
1590.00
1585.00
1580.00
1575.00
0
1
2
3
4
Time
group
j1 c2
1
2

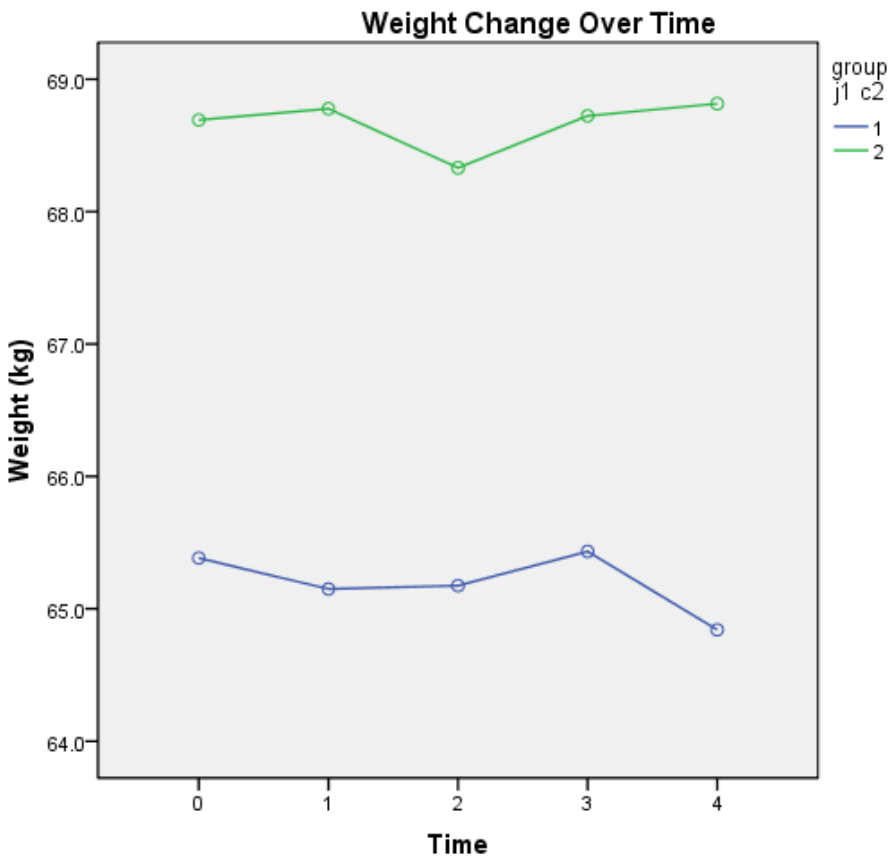

Left SOS Change Over Time
Speed of Sound (SOS)
1590.00
1587.50
1585.00
1582.50
1580.00
1577.50
0
1
2
3
4
Time
group
j1 c2
1
2

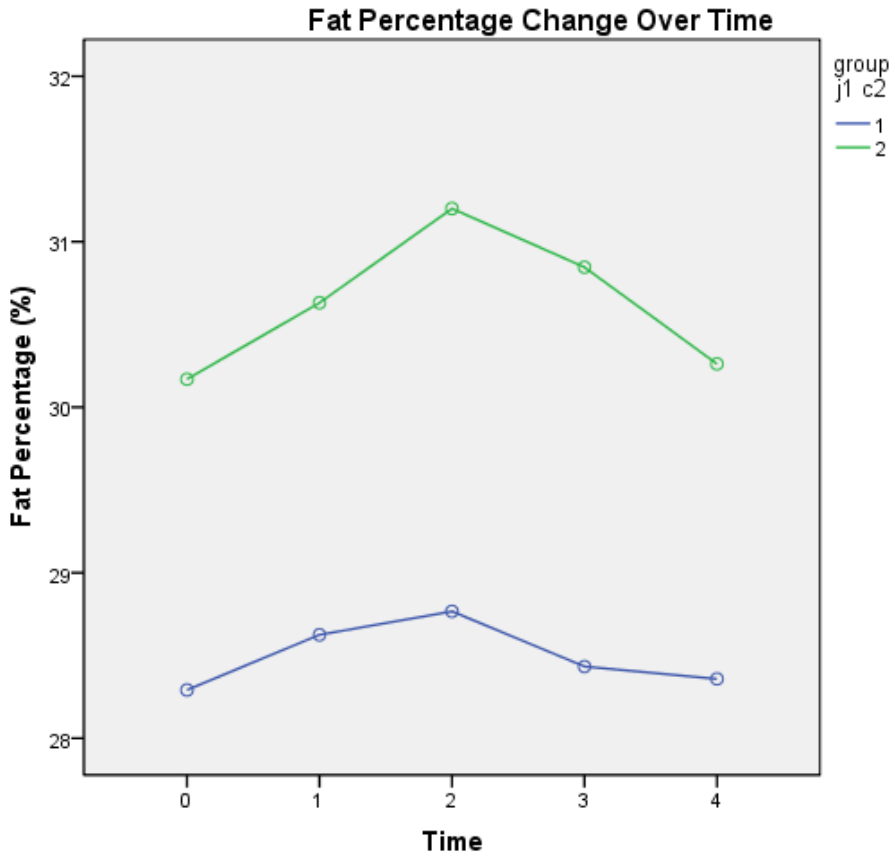

Right BUA Change Over Time
Broadband Ultrasound Attenuation (BUA) (m/s)
124.0
123.0
122.0
121.0
120.0
119.0
118.0
0
1
2
3
4
Time
group
j1 c2
1
2

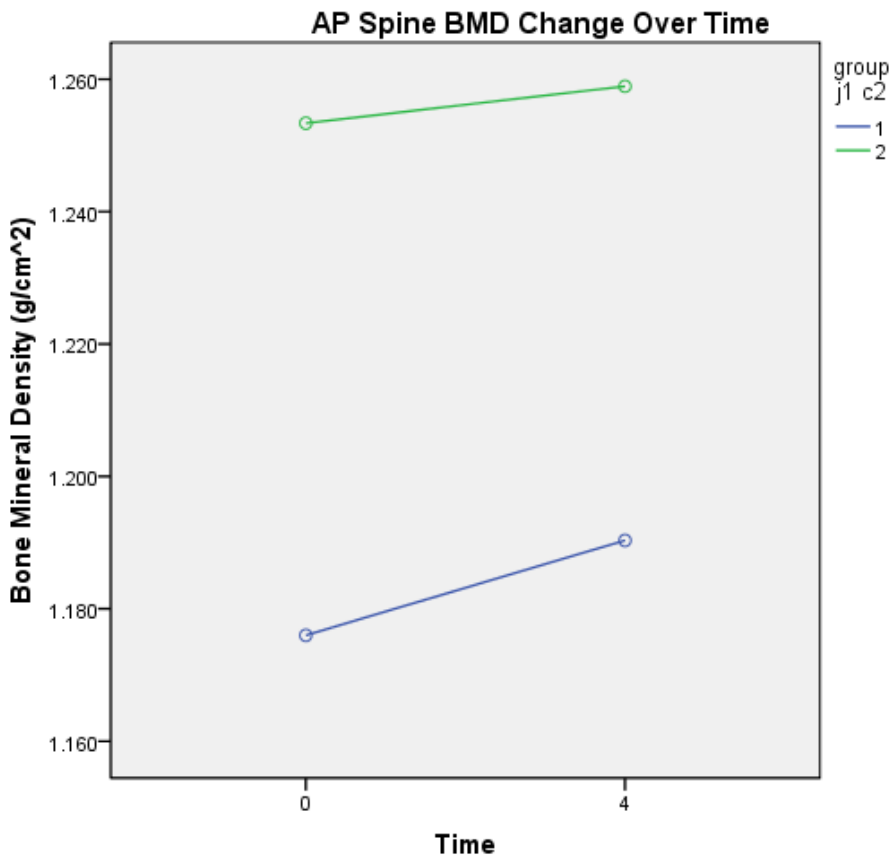

Left BUA Change Over Time
Broadband Ultrasound Attenuation (BUA) (m/s)
126.00
124.00
122.00
120.00
118.00
116.00
0
1
2
3
4
Time
group
j1 c2
1
2

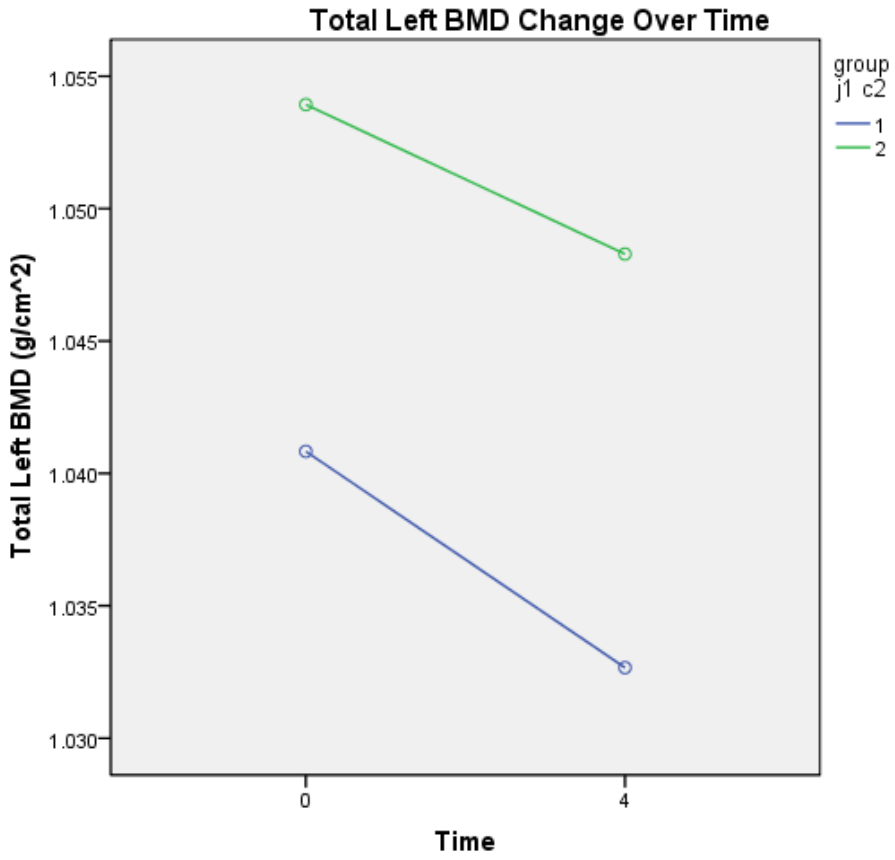

Fat Mass Change Over Time
group
j1 c2
1
2
22.0
21.0
20.0
19.0
Fat Mass (kg)
0
1
2
3
4
Time

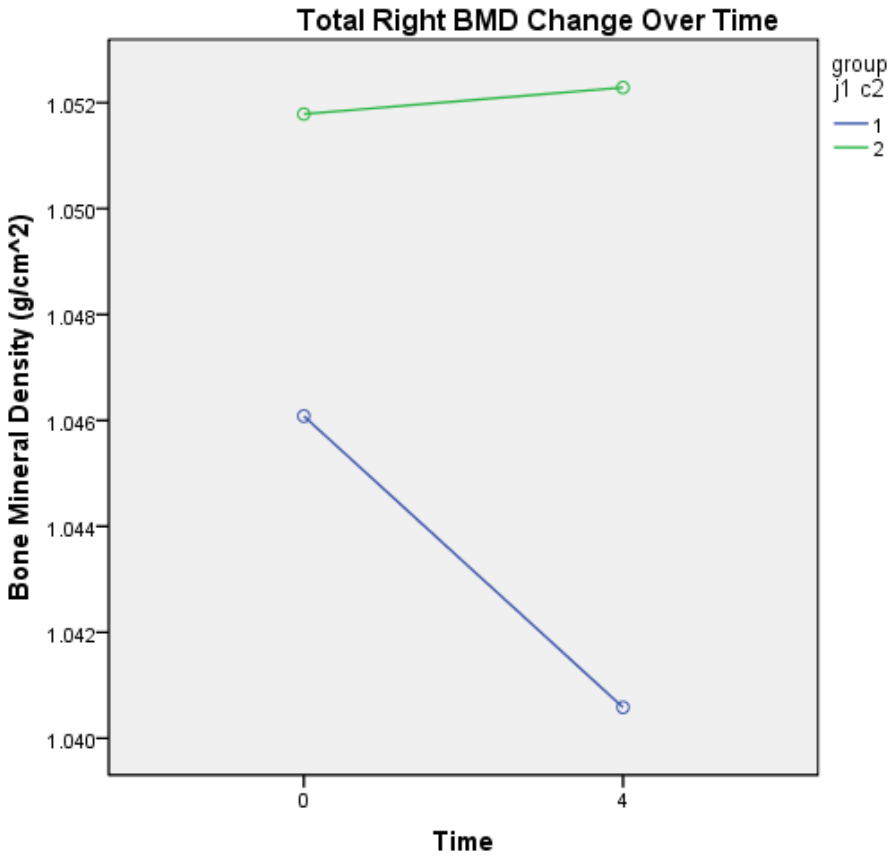

Fat Free Mass Change Over Time
Fat Free Mass (kg)
47.5
47.0
46.5
46.0
45.5
45.0
0
1
2
3
4
Time
group
j1 c2
1
2

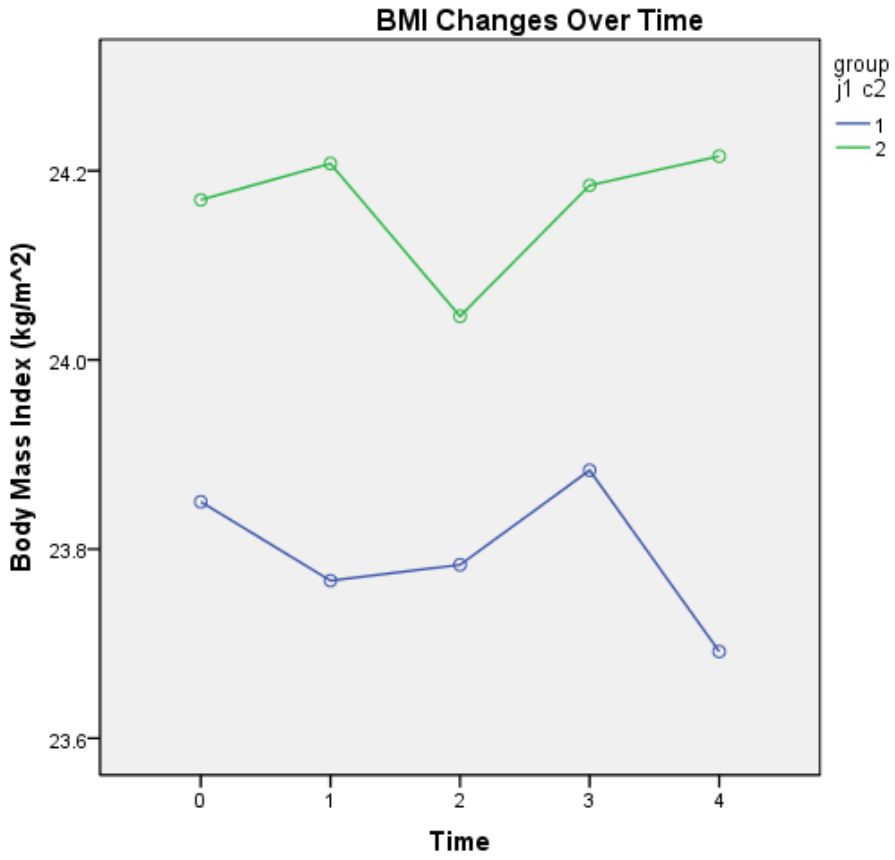

Weight Change Over Time
Weight (kg)
69.0
68.0
67.0
66.0
65.0
64.0
0
1
2
3
4
Time
group
j1 c2
1
2

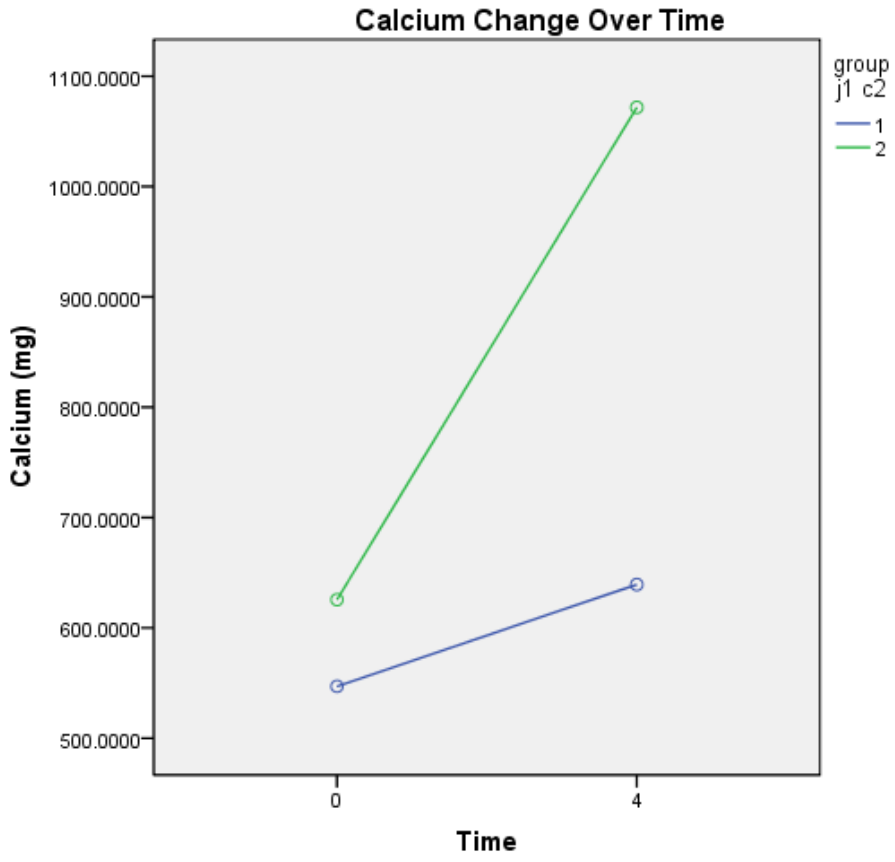

Fat Percentage Change Over Time
Fat Percentage (%)
32
31
30
29
28
0
1
2
3
4
Time
group
j1 c2
1
2

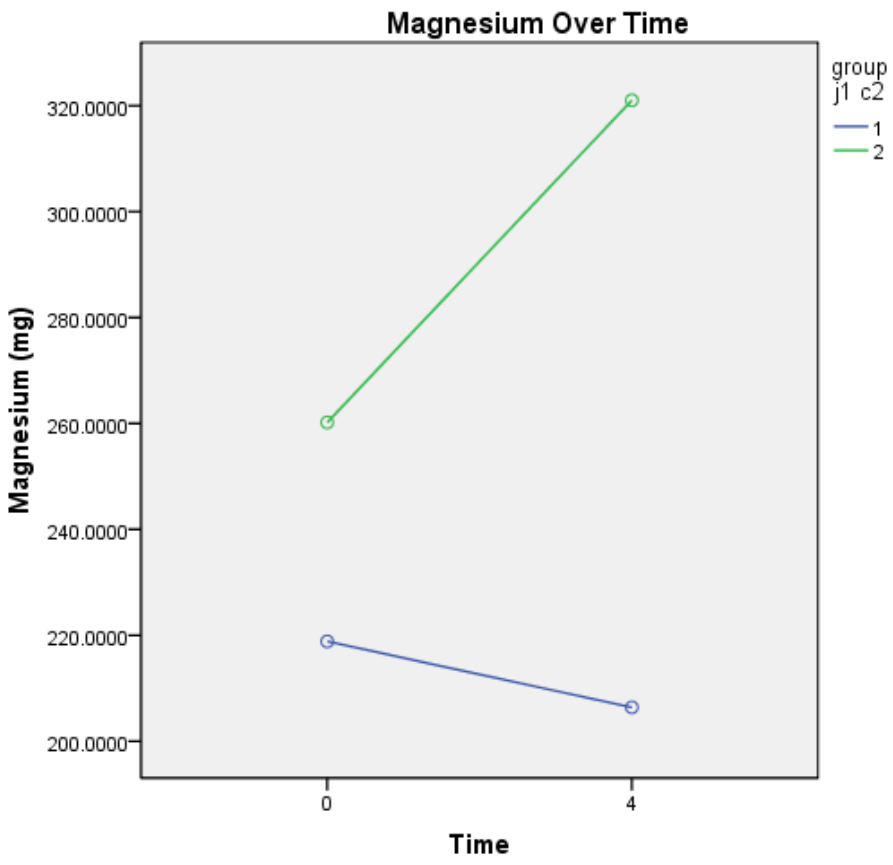

AP Spine BMD Change Over Time
Bone Mineral Density (g/cm^2)
1.260
1.240
1.220
1.200
1.180
1.160
0
4
Time
group
j1 c2
1
2

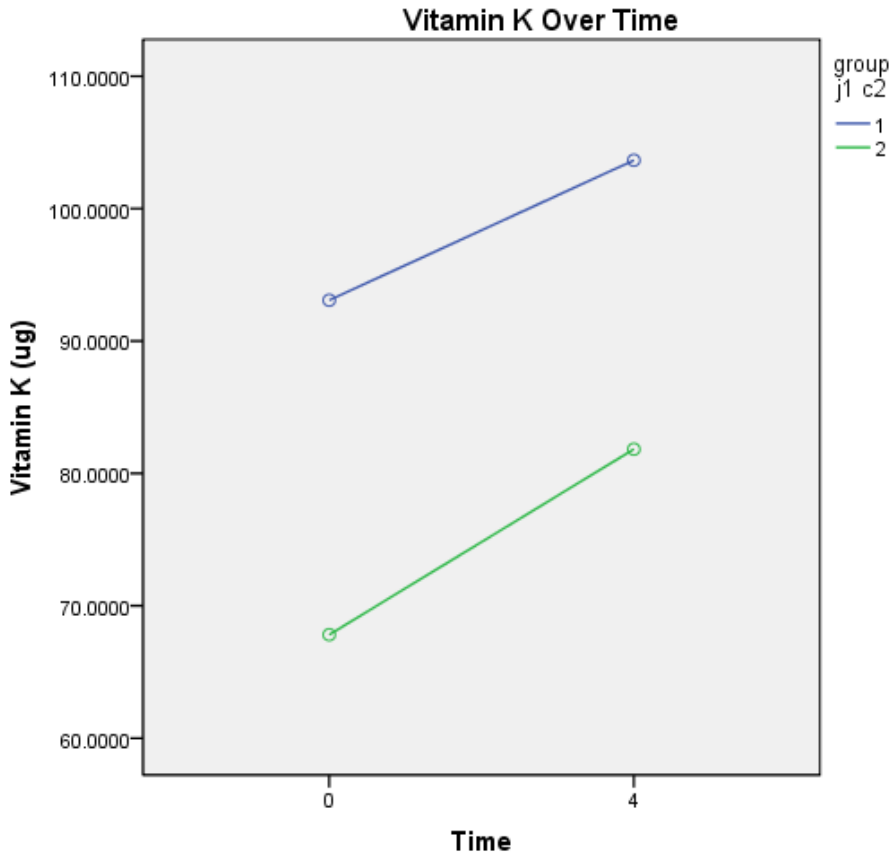

Total Left BMD Change Over Time
Total Left BMD (g/cm^2)
1.055
1.050
1.045
1.040
1.035
1.030
0
4
Time
group
j1 c2
1
2


Total Right BMD Change Over Time
Bone Mineral Density (g/cm^2)
1.052
1.050
1.048
1.046
1.044
1.042
1.040
0
4
Time
group
j1 c2
1
2


BMI Changes Over Time
group
j1 c2
1
2
Body Mass Index (kg/m^2)
24.2
24.0
23.8
23.6
0
1
2
3
4
Time


Calcium Change Over Time
1100.0000
1000.0000
900.0000
800.0000
700.0000
600.0000
500.0000
Calcium (mg)
0
4
Time
group
j1 c2
1
2


Magnesium Over Time
group
j1 c2
1
2
320.0000
300.0000
280.0000
260.0000
240.0000
220.0000
200.0000
Magnesium (mg)
0
4
Time


Vitamin K Over Time
110.0000
100.0000
90.0000
80.0000
70.0000
60.0000
Vitamin K (ug)
0
4
Time
group
j1 c2
1
2

CURRICULUM VITAE

Kristin Joelle Heumann

Arizona State University
7350 East Unity Avenue
Mesa, Arizona 85212
https://sites.google.com/site/kristinheumann/

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Chandler, AZ 85225
(480) 206-8637
kristin.heumann@asu.edu

EDUCATION

Doctor of Philosophy, Physical Activity, Nutrition & Wellness
Arizona State University
Department of Exercise & Wellness, Mesa, Arizona — Expected May 2011
Dissertation: "The Response of Osteocalcin and Ultrasound to Exercise"
Committee Members: Pamela Swan, Chair; Brent Alvar; Linda Vaughan; Jack Chisum

Master of Science, Exercise & Wellness
Arizona State University
Department of Exercise & Wellness, Mesa, Arizona — May 2008
Thesis: "Os Calcis Stiffness Index in Jump Ropers and Normally Active Girls"
Committee Members: Pamela Swan, Chair; Carol Johnston; Chong Lee

Bachelor of Arts, Physical Education with a concentration in Fitness Management
Northwestern College — May 2006
Department of Kinesiology, Orange City, Iowa

TEACHING EXPERIENCE

University-Based

Teaching Associate, College of Nursing & Health Innovation
Department of Exercise & Wellness
Arizona State University, Mesa, Arizona — **August 2007 - Present**

EXW 310 – Technology in Physical Activity — Fall 2010, Spring 2011
This class is designed to help the student to learn to incorporate technology into the field(s) of fitness, wellness, and physical activity. In addition, familiarization with statistical procedures and applications are utilized.

EXW 215 – Physical Activity and Healthy Lifestyles — Fall, Summer 2008, Spring, Summer 2010
The purpose of this course is the application of principles of physical activity to personal fitness testing and program planning for people of all ages, and above all, to have fun while doing it! Physical Activity and Healthy Lifestyles is delivered through lecture material available as online videos, textbook readings, a variety of physical activity and health behavior self assessments, and a variety of online quizzes and assessments.

EXW 301 – Concepts of Fitness and Wellness — Fall, Summer 2008, Spring, Summer 2010
This course examines guidelines for achieving health benefits of physical activity and other healthy lifestyles. It is for all students in the university whose major area of study is not Exercise & Wellness. The assumption is that all college graduates can experience the health benefits of appropriate regular physical activity. The purpose of this class is to give the student practical experiential learning and assess knowledge and comprehension of the key concepts presented in the PowerPoint's, videos, and textbook concepts.

EXW 315 – Lab for Exercise Physiology Summer 2010

This course studies human movement with an emphasis on physiological function of the body in response to physical activity and exercise. Hands-on experience within the laboratory is used to teach the students assessment and function at rest and during exercise.

EXW 212 – Instructional Competency Lab: Cardiovascular Fitness Fall 2008-Fall 2009

This theory/hands-on course is designed to help the student learn various safe and effective teaching methods and modalities that are appropriate for individuals as well as various age groups and physical abilities. This course provides the student with a basic understanding of the effects of cardiorespiratory exercises and general scientific principles relative to improving cardiorespiratory fitness. Specific core competencies are identified and addressed to provide the student with greater knowledge of requirements for various certifications.

EXW 105 – Aerobics Fall 2007-Spring 2008

This course is designed to introduce the student to aerobic group exercise class through a variety of types and styles of movement. This course covers both hi/lo impact activities including walking/jogging routines, circuit training, step aerobics, kickboxing, jump roping, and power exercise. Props necessary for these activities such as hand weights, jump ropes, stability balls, mats, steps, and bands will be utilized.

EXW 105 – Weight Training Fall 2007-Spring 2008

This course is designed to introduce the student to weight training through a variety of modalities involved with effective weight training including free weights, resistance equipment, bands, tubing, stability balls, and one's own body weight. This course covers how to safely use weight training in an exercise program, which muscles are being utilized in which exercises, weight training terminology, and how to design an exercise program.

Teaching Assistant, School of Health Sciences
Universidad Europea de Madrid, Madrid, Spain **January 2010 – May 2010**

Practicum – Physical Fitness & Health Spring 2010

This hands-on course is designed to introduce students to physical fitness assessments and how to assess the client.

Aging and Older Adults Spring 2010

This course is designed to help the students learn how to appropriately develop exercise programs for the aging adult population. Lecture and translation of research articles are utilized to familiarize students with recommendations specific to this population.

Masters Lecturer, EUROSPORT Masters in Multimedia Sports Journalism
Universidad Europea de Madrid, Madrid, Spain **February 2010**

Physical Activity Sciences

This introductory class is designed to introduce journalism students to the exercise science, sport, and health field. Information is provided to students to educate them on health benefits of physical activity, appropriate sport programming, and assessment techniques utilized in the exercise science field.

Community College-Based

Adjunct Faculty, Departments of Physical Education, Health Science, and Exercise Science
Chandler Gilbert Community College, Chandler, Arizona **August 2008 - Present**

EXS 212F – Instructional Competency Lab: Flexibility — Fall 2010, Spring 2011
This is a hands-on course designed to teach the student how to safely and effectively instruct a wide variety of flexibility exercises one-on-one, and to groups of adults of varying ages and physical abilities. This course will cover fundamentals of participant screening, proper warm-up and cool-down, instruction of flexibility exercises, and group instruction skills. The course will address a significant number of core competencies identified for the ACSM Health Fitness Instructor Certification, as well as the NSCA Certified Strength and Conditioning Specialist and Certified Personal Trainer examinations.

PED 117 – Weight Training — Fall 2008, Fall, Spring 2009, Fall 2010, Spring 2011
Fitness activity and wellness study to help develop a lifetime of regular exercise, stress management, and proper nutrition. Workout includes warm-up, aerobic exercise, selected strength exercises, and cool down.

HES 100 - Healthful Living — Spring 2009, Spring 2011
This class is designed to help the student to learn the facts about personal health, wellness, and physical activity, to become an informed health, wellness, and exercise consumer, and to plan a personal lifetime health and wellness program. It is for all "first year" athletes at the college regardless of major area of study. The assumption is that all college graduates can experience the health benefits of appropriate regular physical activity. The ultimate goal is to help the student plan for a lifetime of health, wellness, and physical activity.

PED 115 – Lifetime Fitness — Fall 2008, Spring 2009
Fitness activity and wellness study to help develop a lifetime of regular exercise, stress management, and proper nutrition. Workout includes warm-up, aerobic exercise, selected strength exercises, and cool down.

EXS 212C - Instructional Competency Lab: Cardio — Fall 2008
This theory/hands-on course is designed to help the student learn various safe and effective teaching methods and modalities that are appropriate for individuals as well as various age groups and physical abilities. This course provides the student with a basic understanding of the effects of cardiorespiratory exercises and general scientific principles relative to improving cardiorespiratory fitness. Specific core competencies are identified and addressed to provide the student with greater knowledge of requirements for various certifications.

TEACHING INTERESTS

Sociocultural Aspects of Exercise & Sports
Physical Fitness Assessments
Fitness Management
Exercise Testing
Concepts of Physical Fitness
Cardiovascular Fitness Training
Strength and Conditioning Training
Exercise Prescription

RESEARCH EXPERIENCE

Research Assistant, "ASUKI-Step" Project, Arizona State University Polytechnic, 2009
Barbara E. Ainsworth, Professor

Volunteered to assist with data collection including: measuring height, weight, blood pressure, quantitative ultrasound, waist girth, waist diameter, and the Astrand submaximal bicycle ergometry test.

Research Assistant, "Effects of Combat on Physical Fitness and the Influence on Utilization of Medical Resources," Arizona State University Polytechnic, 2009-2010
Bradley Warr, PhD candidate

Volunteered to assist with data collection including: measuring height, weight, body composition using both bio-electrical impedance and Bod Pod, VO_2max with a modified Bransford and Howley Protocol, 2-minute push-up max and sit-up max tests, bench press and squat one-repetition max test.

Research Assistant, "Comparison of Total Body Water in High School Wrestlers Using Bio-Impedance Measures," Arizona State University Polytechnic, 2008
Chris Keating, MS

Volunteered to assist with data collection including: measuring height, weight, body composition using both bio-impedance spectroscopy and multi-frequency bio-electrical impedance, and hydration status.

RESEARCH INTERESTS

Quantitative ultrasound of the calcaneus in response to exercise across the lifespan
The effects of jump roping on health indices
The effects of Zumba exercise on health and psychological indices

GRANT ACTIVITY

Internal Grants

2010-2011	Arizona State University Graduate and Professional Student Association Research Grant Competition Award – $2000
2010-2011	Arizona State University Charles Corbin Research Fellowship - $500
2010	Arizona State University Graduate and Professional Student Association Travel Award - $750
2009-2010	Arizona State University Graduate and Professional Student Association Research Grant Competition Award - $750
2007-2008	Arizona State University Graduate and Professional Student Association Research Grant Competition Award - $1900
2007	Arizona State University Charles Corbin Research Fellowship - $500

External Grants

2010	Amateur Athletic Union Jump Rope Division Grant for Research - $2000
2010	ACSM Foundation – Doctoral Student Research Grant – *($5000) Not-funded*

PROFESSIONAL PRESENTATIONS

Heumann, K.J., Warr, B.J., Swan, P.D. (June, 2011). Feasibility of measuring acute OCSI changes in 2 exercise groups. To be presented at the *58th Annual American College of Sports Medicine Meeting,* Denver, Colorado.

Warr, B.J., Alvar, B., Dodd, D., **Heumann, K.J.,** Mitros, M., Keating, C., Swan, P.D. (May, 2011). How does combat effect fitness? An evaluation of deployed Arizona National Guardsmen. To be presented orally at the *58th Annual American College of Sports Medicine Meeting,* Denver, Colorado.

Heumann, K.J., Swan, P. (October, 2010). Feasibility of measuring acute changes in os calcis stiffness index following whole-body vibration with resistance and jump training in young women. Orally presented for the student research award competition at the *Southwest Chapter of the American College of Sports Medicine Annual Meeting,* San Diego, California.

Heumann, K.J., Warr, B., Swan, P. (June, 2010). Body composition and the relationship to strength and power. Poster presented at the *57th Annual American College of Sports Medicine Meeting,* Baltimore, Maryland.

Keating, C.J., Swan, P., **Heumann, K.J.** (June, 2010). Comparison of total body water in high school wrestlers using bio-impedance measures. Poster presented at the *57th Annual American College of Sports Medicine Meeting,* Baltimore, Maryland.

Heumann, K.J., Swan, P. (May, 2010). Feasibility of Measuring Acute Changes in Os Calcis Stiffness Index Following Whole Body Vibration With Resistance and Jump Training in Young Women. Poster presented at the *International Osteoporosis Foundation World Congress on Osteoporosis,* Florence, Italy.

Herrmann, S., **Heumann, K.J.,** Bowles, H., Meckes, N., Ainsworth, B. (May 2010). Evaluation of the Global Physical Activity Questionnaire (GPAQ). Poster presented at the *International Congress for Physical Activity and Health*, Toronto, Canada.

Heumann, K.J., Swan, P.D., Ainsworth, B., Yngve, A. (October, 2009). Comparison of bone strength in adults classified by ACSM physical activity guidelines. Poster presented at the *Southwest Chapter of the American College of Sports Medicine Annual Meeting,* San Diego, California.

Herrmann, S., **Heumann, K.J.,** Bowles, H., Ainsworth B. (October, 2009). Validity of the Global Physical Activity Questionnaire (GPAQ). Poster presented at the *Southwest Chapter of the American College of Sports Medicine Annual Meeting*, San Diego, California.

Keating, C., Swan, P., **Heumann, K.J.** (October, 2009). Comparison of total body water in high school wrestlers using bio-impedance measures. Poster presented at the *Southwest Chapter of the American College of Sports Medicine Annual Meeting,* San Diego, California.

Heumann, K.J., Swan, P.D., Kahl, K. (May, 2009). Effects of varying sports and normal activity in pre-pubescent 10-year old girls. Thematic poster presented at the *56th Annual American College of Sports Medicine Meeting*, Seattle, Washington.

Heumann, K.J., Swan, P.D. (November, 2008). A comparison of calcaneal ultrasound measurements in competitive jump ropers and normally active females. Poster presented at the *Southwest Chapter of the American College of Sports Medicine Annual Meeting*, San Diego, California.

Heumann, K.J., Swan, P.D. (February, 2008). Comparison of Calcaneal Ultrasound in Competitive Jump Ropers and Age Matched Controls. Poster presented at the *Building Healthy Lifestyles Conference*, Arizona State University, Mesa, Arizona.

PROFESSIONAL PUBLICATIONS

Submit, Pending Response

Heumann, K.J., Swan, P.D. Os calcis stiffness index values in jump ropers and normally active girls.

Chulvi-Medrano, I., **Heumann, K.J.,** Masia-Tortusa, L., Llopis-Goig, D., Garcia, S. Perceptions of anti-obesity medications among Spanish personal trainers.

Herrmann, S.D., **Heumann, K.J.,** Bowles, H., Ainsworth, B.A. Validity of the global physical activity questionnaire.

Warr, B.J., Dodd, D., Alvar, B., Swan, P.D., **Heumann, K.,** Mitros, M., Keating, C. How do they compare?: an assessment of pre-deployment fitness in the Arizona National Guard.

Warr, B.J., Alvar, B., Dodd, D., **Heumann, K.,** Mitros, M., Keating, C., Swan, P.D. Determining the effects of long-term combat deployments on physical fitness in Arizona National Guard Men and Women.

COMMUNITY PRESENTATIONS

2009	In-Service at Arizona State University, Mesa, AZ "Jump Rope Instruction Training" for Physical Educators
2008	Seminar at Freescale Semiconductor, Tempe, AZ "Diabetes: Care and Prevention"
2007	Seminar at Freescale Semiconductor, Tempe, AZ "The History and Benefits of Jump Rope for Health"

PROFESSIONAL EXPERIENCE

2007-2008	Senior Lifestyle Coordinator, Freescale Semiconductor, Tempe, Arizona Handled new member registration, planned recreation events, performed fitness assessments, taught group exercise classes, and personal trained members
2007	Jump Rope Instructor, Rancho Solano, Gilbert, Arizona Taught elementary school students in an after school jump rope program. Developed plans for the program and interacted individually with students.
2006	Personal Trainer, Arizona State University, Mesa, Arizona Instructed research subjects on how to properly perform exercises.

2006	Administration Specialist, Grand Canyon State Games, Tempe, Arizona Entered participant registration into database, collected and accounted for money received, worked with commissioners to prepare and organize volunteers for each sport event, and prepared registration and athlete check-in for event day.
2005	Intern, Grand Canyon State Games, Tempe, Arizona Entered participant registration into database, attend sporting events, and organize registration information for each sport site.
2004-2006	Fitness Instructor, Northwestern College, Orange City, Iowa Instructed group fitness including: step aerobics and cardio jump rope.
2003-2004	Fitness Instructor, Fort Lewis College, Durango, Colorado Instructed group fitness: cardio jump rope.

CERTIFICATIONS

2010-Present	Zumba Licensed Instructor
2009-Present	American College of Sports Medicine Health Fitness Specialist
2006-Present	American Red Cross CPR/AED Professional Rescuer
2003-Present	Coaching Certification: American Sport Education Program

PROFESSIONAL SOCIETY MEMBERSHIPS

American College of Sports Medicine
National Strength and Conditioning Association
American Alliance of Physical Education, Recreation, and Dance
Arizona Chapter, American Alliance of Physical Education, Recreation, and Dance

SERVICE

Academic

2007-Present	Building Healthy Lifestyles Conference Registration Committee, Vice-President & President of Building Healthy Lifestyles Student Organization, Arizona State University, Mesa, Arizona
2007-Present	President: ASU Exercise and Wellness Graduate Club, Arizona State University, Mesa, Arizona
2009	Guest Lecturer, EXW 450 – Cultural and Social Issues in Exercise and Wellness, Arizona State University, Mesa, Arizona
2009	Writing Group Studio Leader, Arizona State University, Mesa, Arizona
2006	Teaching Assistant, EXW 425 – Exercise Prescription, Arizona State University, Mesa, Arizona
2004-2006	President & Vice-President: Kinesiology Klub, Northwestern College, Orange City, Iowa

Community

2010-Present	Member: Women's Auxiliary Board for Improving Chandler Area Neighborhoods
2007-Present	Co-Commissioner & Commissioner of Jump Rope: Grand Canyon State Games, Tempe, Arizona

2007-Present	Volunteer for the Grand Canyon State Games, Summer, Winter, and Native American Games, Winners Circle Weekend, Tempe, Arizona
2008-Present	Annual Volunteer Events: Frank Kush Family Fun Run and Dog Walk, Tempe, Arizona Relay for Life, Mesa, Arizona

AWARDS

2010	Gail Butterfield Award Recipient, Southwest American College of Sports Medicine Student Research Award Competition, San Diego, California
2006	Physical Education Major of the Year Award, Northwestern College, Orange City, Iowa
2004	Amateur Athletic Union Major Contributor to the Sport of Jump Rope, Des Moines, Iowa
2002	Joe Selleh Award, Tempe, Arizona