UNIVERSITY OKLAHOMA

GRADUATE COLLEGE

EFFECTS OF YOGA EXERCISE ON BONE DENSITY AND BONE METABOLISM IN PREMENOPAUSAL WOMEN

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

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Norman, Oklahoma
2010

EFFECTS OF YOGA EXERCISE ON BONE DENSITY AND BONE METABOLISM IN PREMENOPAUSAL WOMEN

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ACKNOWLEDGMENTS

I am grateful that I have people in my life that make me want to be a better person. I think first of my Mom. She always trusts and respects my decisions. Without her continued support and prayers, I could not have had the 4.5 year long journey in Oklahoma. One of the happiest moments in my entire life was with my Dad when I got accepted into college. I still remember his tears and tremendous love. At this moment Dad up in heaven, I believe that you must be so proud of your youngest daughter. You are always in my heart and I love you. To my two older sisters, brothers-in-law, uncle, and lovely nieces and nephews, you guys make me feel happy and strong everyday. Thanks to you guys, I did not feel lonely in the United States.

It has been a great privilege to spend 4.5 years in the Department of Health and Exercise Science. I would like to thank everyone in the Department for continuing to be friendly and supportive during my academic years. I felt always at home and I will never forget memories I had in the Department. I would like to thank my committee Dr. Debra Bemben, Dr. Michael Bemben, Dr. Allen Knehans, Dr. Mark Anderson, and Dr. Rosemary Knapp for their knowledge, encouragement, and insight throughout the course of my research. Their academic support and personal cheering made it possible to finish up my 8 month long journey. I would also like to give a heartfelt, huge thanks to Dr. Debra Bemben, who continuously encouraged me to move on and allowed me to see my potential ability. I felt like I was riding the roller coaster during the academic years, which was sometimes exciting, challenging, and scary. However, one day I realized that she was always there for me to share in the hard time I was having and to encourage me not to give up. Thank you Dr. Deb for believing in me. I know this is not the end but the beginning of my future career. When I get to the point where I have students, I will follow your academic philosophy.

Finally, I want to thank the women who participated in my 8 month Yoga training study. Without their dedication, I would not have done this study. For 8 months, I was so happy having fresh morning air, listening to soothing music, doing Yoga, and watching sunrises with you all together. These experiences truly make my studies worthwhile.

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ABSTRACT

Although the beneficial effects of Yoga for increasing psychological well-being and decreasing stress and reducing cardiovascular risks have been reported, no studies to date have examined intervention Yoga training to investigate its effects on bone metabolism and hormone responses. **PURPOSE:** The purpose of this study was to examine the effects of an 8 month Yoga exercise program on bone biomarkers, areal bone mineral density (aBMD) for the total body, dual proximal femur, and lumbar spine and volumetric bone mineral density (vBMD) for the tibia and forearm in premenopausal women. A secondary purpose was to examine IGF-I, body composition, and muscular strength changes. **METHODS:** Thirty-four healthy women were randomly assigned either to a Yoga group (YE, n=16, mean age; mean±SD, 45.73±3.82) or a control group (CON, n=18, 43.22±4.16). Subjects in YE group performed 60 minutes of an Ashtanga Yoga series two times per week with one day between sessions for 8 months in morning sessions (6:30 - 7:30 am, Mondays/Wednesdays). Each Yoga session consisted of 15 minutes of warm-up exercises, 35 minutes of Ashtanga Yoga postures and 10 minutes of cool-down with relaxation. Session intensity was progressively increased during the 8 months. Subjects in CON group did not receive the Yoga exercise intervention, and they were encouraged to maintain their normal daily lifestyle monitored by the bone-specific physical activity questionnaire at 2 month intervals for 8 months. Bone formation (bone alkaline phosphatase, Bone ALP) and bone resorption (tartrate-resistant acid phosphatase, TRAP5b) were assessed at baseline and after Yoga intervention. aBMD and vBMD were measured using DXA and pQCT, respectively. Body composition (DXA) and muscle strength (1RM) for leg press (LP), knee extension (KE), knee flexion (KF), lat pull down (LPD), shoulder press (SP), and biceps curl (BC) were

measured. **RESULTS:** There were no significant differences between the groups in age, height, and weight at baseline. A significant group x time interaction was detected in serum Bone ALP ($p<0.01$). Paired samples t-tests indicated that the CON group had a significant decrease in serum Bone ALP concentration. There were also significant group differences ($p<0.05$) in percent changes for Bone ALP as the YE group had a significantly greater relative increase compared to the CON group (9.1% vs. -7.1%). However, no significant changes were found in TRAP5b in either group after the 8 month Yoga training intervention. There were also no significant group differences ($p>0.05$) in percent changes for TRAP5b and ratio of Bone ALP to TRAP5b, but there was a trend ($p=0.061$) for serum TRAP5b, which increased slightly in YE group whereas it decreased in CON group (1.0±4.6% vs. -8.3±6.5%). No significant BMD differences and percent changes were detected in the total body, lumbar spine, and dual femur sites between groups ($p>0.05$). However, a group x time interaction was detected ($p<0.05$) as the radius 33% in YE slightly decreased whereas it increased slightly in the CON group ($p<0.05$). There were no significant changes in bone content, bone area, cortical thickness, periosteal circumference, and SSI for total, cortical and trabecular at the tibia 4% and 38% ($p>0.05$). However, a significant time effect was detected ($p<0.01$) for cortical vBMD at the tibia 66% site as this variable decreased significantly in both groups after the 8 month intervention (-0.3±0.1, YE vs. -0.1±0.1, CON). For the radius 4% and 66%, no changes were detected in vBMD variables except for the trabecular vBMD, which decreased significantly in both groups ($p<0.05$). The trabecular vBMD of the radius 4% in percent changes were -4.0±2.4 for the YE group and -2.6%±2.4 for the CON group. A significant time effect was observed ($p<0.01$) as serum IGF-I levels decreased in both groups after the 8 month intervention (-17.2±4.3%, YE vs. -6.2±4.2%, CON). However, no group x

time interaction was detected ($p>0.05$). No significant differences and percent changes in weight, total fat mass, and %fat were detected ($p>0.05$). However, bone free lean body mass in both groups significantly increased ($p<0.01$); bone free lean body mass in the YE group increased slightly more compared to the CON group (2.1±0.7%, YE vs. 1.9±0.8%, CON). The 8 month Yoga training intervention significantly ($p<0.01$) increased LP strength in the YE group ($p<0.01$), whereas LP strength decreased slightly in the CON group. There were also significant group differences ($p<0.01$) in percent changes for SP as the YE group had a significantly greater relative increase compared to the CON group (7.0±10.7%, YE vs. -2.1±2.7%, CON). However, after adjusting baseline mean differences for SP, one-way ANCOVA did not detect group differences ($p>0.05$). Also, two-way mixed factorial ANOVA with repeated measures detected a significant time effect ($p<0.05$) for LPD strength, which significantly decreased LPD muscle strength in both groups. There were no time or group x time interaction effects were observed for BC, KE, and KF ($p>0.05$) after the 8 months. **CONCLUSION:** The findings of this study suggest that the 8 month Ashtanga Yoga was able to generate sufficient mechanical loading forces to elicit increases in bone formation (Bone ALP) as well as increases in leg press muscle strength in premenopausal women.

CHAPTER I

INTRODUCTION

Yoga is an ancient Indian practice referring to traditional physical and mental disciplines, established in India around 2500 B.C.(77). The Sanskrit word, Yoga, means “union” or “yoking” and has been defined as the union of mind and body, heart and actions (66). Of all Yoga types, asanas, physical postures, are well known in most countries and this form of Yoga is referred to as Hatha Yoga (66). There are many different types of Yoga to practice in terms of breathing exercises, postures, and spirituality. Hatha Yoga is more likely to be a slow-paced stretching, whereas Ashtanga Yoga is a vigorous and athletic style of practice (52). Yoga has various and creative asanas, including breathing techniques (pranayama) and meditation (dhyana). According to the 2008 Yoga in America Market Study (1), 15.8 million Americans have practiced Yoga with 40.6% in the 18 to 34 age range; 41% between 35 to 54 years old; and 18.4% being over 55 (1). Among the participants, 72.2% are women and 27.8% are men (1).

Due to its physical and mental benefits for individuals, Yoga is becoming a popular physical activity in modern western society. Previous studies have shown increases in muscular endurance (101), flexibility (40), and maximal oxygen uptake (VO_{2max}) (8). Similarly, Tran et al. (130) reported that Yoga four times per week for 8 weeks significantly increased muscle strength and endurance, flexibility, and cardiorespiratory endurance in 10 untrained subjects (nine females and one male). For health benefits, Yoga may be an invaluable alternative for individuals who cannot participate in vigorous exercises such as high intensity weight bearing exercise and fast running. In addition, Yoga may provide increased motivation, enjoyment, and feelings of accomplishment by performing various asanas each session. As previously reported (130), such health benefits may inspire people to maintain daily physical

activity with Yoga. However, the exercise intensity may not satisfy the recommendations by the American College of Sports Medicine (ACSM), which suggests that adults aged 18-65 years old need moderate intensity aerobic activity for a minimum of 30 minutes five days a week or vigorous intensity for a minimum of 20 minutes three days a week to promote and maintain health (51). Hagins et al. (49) investigated whether a Yoga practice using various postures meets the current recommendations for levels of physical activity required to improve and maintain health and cardiovascular fitness. They reported that the metabolic costs of Yoga averaged across the entire session were similar to walking on a treadmill at 2 mph; therefore, Yoga did not meet the ACSM recommendations for improving or maintaining health or cardiovascular fitness. They also suggested that increasing the portion of sun salutation postures may partially contribute to improving cardio-respiratory fitness.

Although Yoga has various health benefits, its effects on bone have not been examined. Osteoporosis is a progressive disorder of compromised bone strength characterized by low bone mineral density (BMD), high bone turnover, and microarchitectural deterioration of bone tissue (75). The process of bone loss and bone formation is mediated at the cellular level by osteoclasts, which act to remove old bone, and osteoblasts, which are responsible for bone formation. BMD decreases with age and there is a particularly noticeable decline in women approaching the menopausal period due to the pronounced drop in estrogen levels (58). BMD is used to determine the severity of bone loss and fracture risk and to distinguish patients with osteoporosis from those with osteopenia and normal bone density. Dual energy X-ray absorptiometry (DXA) is the gold standard for BMD assessment and the diagnosis of osteoporosis. The World Health Organization (WHO) categorizes osteoporosis in

postmenopausal women as a BMD of at least 2.5 standard deviations below the mean BMD of young adult women (a T-score ≤ -2.5) (92).

Recently, peripheral Quantitative Computed Tomography (pQCT) has increasingly been applied to quantitative assessment of the skeleton in adults and children. Unlike DXA, which measures 'areal' (g/cm^2) BMD, pQCT measures volumetric (mg/cm^3) BMD, so it is not size-dependent, and provides separate measures of cortical and trabecular bone (46). pQCT can discriminate between trabecular and cortical components of bone, which are known to show different responses to aging, various diseases, and treatments (38, 111). Although in some applications pQCT appears to be a more appropriate method than DXA, this does not lessen the clinical value of DXA. On the contrary, pQCT can efficiently supplement the information of DXA by providing details about the cross-sectional geometry and trabecular density of many peripheral skeletal sites (118).

Bone mineral density is maintained through the dynamic process of bone formation and bone resorption, called bone remodeling. Biochemical markers of bone remodeling provide a dynamic measurement of skeletal status (81). Bone metabolic markers such as bone alkaline phosphatase (Bone ALP), a marker of bone formation, cross-linked C-telopeptide of Type-I collagen (CTX), a marker of bone resorption, and Tartrate-Resistant Acid Phosphatase 5b (TRAP5b), a marker of bone resorption have been used to measure bone metabolism (17, 74, 129) and monitor bone remodeling rates (19). In clinical populations, bone metabolic markers are used to provide useful information for dynamic stages of bone turnover as well as monitoring treatment for osteoporosis (50).

Bone remodeling is a complex process involving a number of cellular processes resulting in the coordinated resorption and formation of new bone. Bone

remodeling is regulated by a variety of systemic hormones and by local factors. Growth hormone (GH) plays a crucial role in the maintenance of bone mass in adults by regulating bone remodeling through a complex interaction of circulating GH, insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and locally produced IGFs and IGFBPs, acting in an autocrine and paracrine way on bone tissue (134). IGF-I is a peptide hormone that is synthesized by many peripheral tissues in response to the action of GH and is released to serum in accordance with its rate of synthesis. In bone, it appears to act on osteoblasts and preosteoblasts, stimulating the synthesis of both osteocalcin (OC) and Type I collagen (22). Thus, IGF-I is an important promoter of bone formation and a reduction of its synthesis in bone (22, 128), or a reduced serum concentration (54, 124) are accompanied by reduced bone formation. *In vivo*, Ebeling et al. (30) demonstrated that treatment of normal women with IGF-I activates both osteoclasts and osteoblasts, but with a more prominent effect on bone formation compared to resorption. Other researchers have found similar results, showing that IGF-I stimulates osteoblasts selectively to a greater degree than osteoclasts (48, 59).

The primary hormones involved in regulating blood calcium levels and bone remodeling are parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D_3 (calcitriol). Excess calcium in the blood leads to the release of calcitonin, which causes deposition of calcium in bone. Conversely, when blood calcium levels drop below normal (about 10 mg/dL), PTH stimulates osteoclast activity, causing calcium to be released from the bone. Calcitrol is important for the absorption of calcium from the intestines. It regulates the calcium and phosphorus levels in the blood by promoting their absorption from food in the intestines, and by promoting re-absorption of calcium in the kidneys (44).

According to the ACSM position stand on physical activity and bone health (67), physical activity appears to play an important role in maximizing and attenuating bone loss during childhood and early adult years, respectively, and reducing falls and fractures in the elderly. ACSM recommends that weight-bearing endurance activities, such as tennis, stair climbing, jogging, and at least intermittently during walking, help to preserve bone health during adulthood. The intensity should be moderate to high, in terms of bone-loading forces and performed three to five days per week for weight bearing endurance activities and two to three times per week for resistance exercise (67). It is well-recognized that appropriate overload for improving bone mass must induce forces greater than those experienced by activities of daily living (115). In response to the mechanical forces exerted on the bones, the bone adapts to altered physical stimuli termed bone adaptation and it requires bone cells to detect mechanical signals in the original place and integrate these signals into appropriate changes in the bone architecture (132). Turner (132) proposed three major rules for bone adaptation to mechanical stimuli: 1) bone adaptation is driven by dynamic, rather than static, loading; 2) the mechanical loading period need only to be short in duration and extended durations result in diminishing returns; and 3) bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals.

Numerous exercise intervention studies have reported that the mechanical load from exercises that generate relatively high ground-reaction and joint reaction forces (resistance training, plyometrics designed to produce fast, powerful movements) acts to increase BMD in femoral neck and/or lumbar spine in sedentary women (10, 11, 79). However, differences in BMD responses are observed depending on the type of exercise. Previous studies have indicated that weight-bearing exercises positively

affect bone metabolism, and biochemical markers have been used to evaluate the mechanisms involved (17, 70, 83). High-intensity progressive resistance training was shown to be effective for increasing BMD at the lumbar spine but not the femoral neck in premenopausal women (85). Shibata et al. (117) reported the influence of one year of walk training and walk and jump training on BMD in premenopausal women. They found no significant decrease in BMD, despite a significant decrease in body weight, whereas numerous studies have reported that a decrease in body weight leads to decreased BMD (29, 103). Also, Bone ALP was significantly increased after one year of walk and jump training. Jumping exercises that increased hip BMD of premenopausal women were ineffective in postmenopausal women who were not on hormone replacement therapy (11).

Among non-traditional forms of exercise, Tai Chi (a traditional Chinese martial art) has been increasingly recommended for osteoporotic women as a safe and effective exercise for bone density maintenance. The U.S. Surgeon General's 2004 report on osteoporosis specifically recommends Tai Chi as a good exercise for fall prevention (39). In a cross-sectional study (98), subjects who had been practicing regular Tai Chi exercise for more than 3 years had an overall higher BMD at spine and hip, as well as better neuromuscular function compared to sex- and age-matched sedentary controls. A long-term randomized intervention study (116) found both Tai Chi and resistance training groups had higher levels of serum Bone ALP relative to the baseline and the Tai Chi group exhibited a greater increase in serum Bone ALP than the resistance training group in the elderly. They concluded that Tai Chi is beneficial for increasing bone formation in the elderly.

Recently, Yoga has become a popular activity in the west and many people who participate believe that Yoga has beneficial effects on BMD similar to Tai Chi.

Both Yoga and Tai Chi have similar characteristics in that they involve coordinated movements with deep breathing and they require strong upper and lower body strength and muscle endurance. In Yoga, healthy stresses are applied in a variety of unusual directions such as simple back bending, twist, and bridge poses which help to strengthen the spine and may increase BMD. In addition, Yoga involves dynamic movements, sun salutations, which include jumping in different directions. However, it is not clear whether Yoga is as effective for improving BMD as weight-bearing exercises like running, jumping, or resistance training (67). Results of a cross-sectional study (127) suggested that Yoga did not provide enough of a stimulus to increase BMD at any sites to levels significantly above those seen in inactive women. Although the beneficial effects of Yoga in increasing psychological well-being and decreasing stress (56) and reducing cardiovascular risks (55) have been reported, no studies to date have examined intervention Yoga training studies to investigate their effects on bone metabolism and hormone responses.

Purpose

The purpose of this study was to examine the effects of an 8 month Yoga exercise program on bone biomarkers (Bone ALP and TRAP5b), BMD of the total body, dual femur, lumbar spine, tibia, forearm and bone geometry characteristics in premenopausal women. Subjects were healthy premenopausal women, between the ages of 35 and 50 years, who were randomly assigned to either the Yoga intervention group or to the control group. A secondary purpose was to examine responses of IGF-I, body composition, and muscle strength (1RM) to the Yoga intervention.

Research Questions

1. Will an 8 month Yoga exercise program significantly alter rates of bone formation and/or resorption as measured by the bone markers, Bone ALP and TRAP5b?
2. Will an 8 month Yoga exercise program increase BMD in total body, dual femur, lumbar spine, and forearm sites in premenopausal women compared to the control group?
3. Will an 8 month Yoga exercise program alter bone geometry characteristics measured by pQCT?

Hypotheses

1. An 8 month Yoga intervention will result in increases in serum Bone ALP concentrations and decreases in serum TRAP5b concentrations.
2. The Yoga intervention will result in increases in BMD for total body, dual femur, lumbar spine, and forearm in premenopausal women.
3. The Yoga intervention will alter tibia and radius bone characteristics, specifically, in total volumetric BMD (vBMD), trabecular vBMD, cortical vBMD, and Strength-Strain Index (SSI).

Sub Questions

1. Are there significant changes in resting hormone responses (IGF-I) after an 8 month Yoga exercise program?
2. Are there significant changes in body composition variables (% body fat, fat mass (FM), and bone free lean body mass (BFLBM))?
3. Will an 8 month Yoga exercise program increase muscle strength?

Subhypotheses

1. The Yoga intervention will significantly increase the resting serum concentrations of IGF-I in the Yoga exercise group.
2. The Yoga intervention will result in significant decreases in % body fat and FM, and increases in BFLBM.
3. The Yoga intervention will increase muscle strength in premenopausal women.

Significance of the Study

The significance of this study is to provide premenopausal women an alternative way to increase BMD. Physical activities (high-impact training; jumping and strength training; resistance exercise) are recommended for osteoporosis to maintain or improve BMD at specific sites in pre- and postmenopausal women (10, 11, 79, 144). Alternative activities provide many choices for women who may not be interested in traditionally recommended exercises. In relation to Yoga, previous studies have shown increases in muscular endurance (101), flexibility (40), and maximal oxygen uptake (VO_{2max}) (8). Although Yoga exercise has the potential for improving BMD like Tai Chi does (98, 116), no studies to date have examined intervention Yoga training and its effects on bone health. This study will provide scientific evidence for how Yoga exercise affects bone metabolism and hormone responses and also will investigate whether its stimulus is adequate to improve BMD in premenopausal women.

Assumptions

1. All subjects gave maximal effort during training and testing sessions.
2. All subjects provided accurate information about the medical, menstrual history, health history, and calcium intake.

3. All subjects were in a fasted state at the time of the blood draws for at least 8 hours prior to the study.

Delimitations

1. The findings of this study can only be applied to healthy premenopausal women not taking hormonal birth control between the ages of 35 and 50 years.
2. All subjects were free of any history of chronic back or joint problems and cardiovascular disease.
3. Subjects were not engaged in vigorous exercise programs such as weight lifting for at least 12 months prior to the study.
4. The subjects participated in the Yoga exercise program for 8 months.
5. The subjects were recruited from the University of Oklahoma, Norman campus, and the surrounding area.

Limitations

1. It is plausible that other factors, such as nutrition status and sunlight exposure, contributed to changes in bone markers and in IGF-I.
2. Although participants' outside physical activity was monitored by questionnaires, daily activities performed outside of the training program were not controlled.
3. Although each Yoga technique was precisely instructed in the same manner, the variations of Yoga postures, sequences, and duration of each movement are dependent on the subject's physical abilities.

Operational Definitions

Areal Bone Mineral density (aBMD; g/cm^2): Bone mineral content (g) divided by bone area (cm^2) (46).

Asanas: Postures for creating firmness of body, steadiness of intelligence, and benevolence of spirit. This is the physical practice most familiar to Westerners as Yoga (66).

Body composition: It is used to describe the percentages of fat, bone and muscle in human bodies (53).

Bone Alkaline Phosphatase (Bone ALP): A biochemical marker of bone formation that is an osteoblastic membrane-bound tetramer also found circulating freely in serum. It can be measured in the serum to determine bone formation (65).

Bone Mineral Content (BMC): BMC (mg/mm) in the setting of pQCT analyses represents the mass of mineral per unit of axial bone length. It should be noted that this definition of BMC is different from that used in DXA, where BMC (g) refers to the amount of mineral in all the bone regions studied and thus is in addition influenced by bone length or the size of the region of interest (100).

Bone remodeling: A complex process involving a number of cellular processes directed toward the coordinated resorption and formation of new bone (134).

C-telopeptide of Type-I collagen cross-links (CTX): A bone resorption marker that can be measured in the serum to determine the degradation products of Type I collagen (65).

Dhyana (Meditation): Withdrawing the consciousness into the soul (66).

Dual energy X-ray Absorptiometry (DXA): DXA uses the attenuation of x-rays through bone to measure bone mineral content at a skeletal site. This type of measurement is areal, providing a two-dimensional representation of bone. DXA

scans are primarily used to evaluate BMD and are also used to measure total body composition.

Hatha Yoga: The word hatha is a Sanskrit combination of the world ha (sun) and tha (moon), which is itself a union of opposites. (66).

Insulin-like growth factor I (IGF-I): A polypeptide protein hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults (22).

IGF binding protein 3 (IGFBP3): The most abundant of the IGF binding proteins in blood and the highest affinity for the IGFs (44). A protein that modulates IGF-I activity and serves as a carrier protein for IGF-I (99).

Osteoblast: The cells responsible for formation of bone (44).

Osteoclast: The cells responsible for bone resorption (44).

peripheral Quantitative Computed Tomography (pQCT): pQCT measures volumetric (mg/cm^3) BMD, so it is not size-dependent, and provides separate measures of cortical and trabecular bone (46).

Pranayama: A set of breathing exercises designed to help the yogi master the life force (66).

Premenopausal: Women that have regular menstrual cycles that have not changed recently in length (47).

Regular menstrual cycle: A recurring cycle of physiologic changes that occurs in reproductive-age females (125).

Strength-strain Index (SSI): SSI combines a geometrical parameter of bone strength (section modulus) with a measure reflecting the properties of cortical bone tissue (cortical vBMD). The use of SSI as a parameter of bone strength has been validated in both animal and human studies (100).

Sun salutation: It is a warm-up routine for most Yoga practices and that can serve as a stand alone routine for building stamina, strength, and flexibility (66).

Tai Chi: It is an internal Chinese martial art often practiced for health reasons and also referred to as Tai Chi Chuan.

Tartrate-Resistant Acid Phosphatase 5b (TRAP5b): TRAP5b is typically expressed in proportion to osteoclast activity and is secreted into the circulation (72).

Total Volumetric Bone Mineral Density (Total vBMD): Total vBMD is defined as the ratio between Bone mineral Content (BMC) and the total cross-sectional area of a bone (100).

Trabecular Bone: It is also called spongy bone and found at the ends of the long bones, in the vertebrae, and in the internal portions of the pelvis, skull, and other flat bones (44).

Ujjayi Breathing: Ujjayi, which means "victoriously uprising," is the most common yogic breathing technique. Take a deep inhalation followed by a deep exhalation (66).

Volumetric Cortical Bone Mineral Density (Cortical vBMD): Cortical vBMD represents the density of the solid cortex, reflecting material density and cortical porosity (100).

CHAPTER II

REVIEW OF LITERATURE

Introduction

Osteoporosis is a progressive disorder of compromised bone strength characterized by low BMD, high bone turnover, and microarchitectural deterioration of bone tissue (75). According to a recent report by the United States Surgeon General (39), half of Americans over age 50 will be at risk for fractures from osteoporosis and low bone mass by 2020 if no immediate action is taken by doctors, health systems, policymakers and those at risk. The risk of a fracture is greatest in women and roughly 4 in 10 white women age 50 or older in the United States will experience a hip, spine, or wrist fracture sometime during the remainder of their lives. Particularly for postmenopausal women, the precipitous decline in estradiol levels is accompanied by loss of bone mineral, reduced BMD, and increased risk of osteopenia and osteoporosis (104). Because peak bone mass occurs in approximately in the third or fourth decade of life (102), studying the beneficial effects of physical activity on bone may be more appropriate in premenopausal women to potentially reduce the future risks of fracture or osteoporosis.

Mechanical loading is theorized to be the primary factor to which bone adapts its strength (32) and the loads produced by muscle forces from physical activity result in the largest strains on bone (73). Numerous exercise intervention studies have reported that the mechanical load from exercise that generate relatively high ground-reaction and joint-reaction forces (resistance training and plyometrics) acts to increase BMD in femoral neck and/or lumbar spine in sedentary women (10, 11, 79).

In recent years, specific bone metabolism markers have been developed that reflect rates of bone formation or resorption. These markers are used especially in

understanding the pathologic state and in assessing the effect of treatment for osteoporosis and related diseases (35). One important determinant of bone strength that is not assessed by either BMD or clinical risk factors is the rate of bone remodeling. High levels of bone turnover markers are associated with an increased risk of fracture (105), while reduced bone turnover is associated with therapeutic efficacy of bone resorption inhibitors (25).

The mechanism of circulating IGF-I effects on bone has been extensively studied. In a study by Liu et al. (78), women in the highest quintile for serum IGF-I concentration were the youngest (28.6 ± 0.4 years), and their BMD was the highest, indicating that women with a higher serum IGF-I level are less likely to develop osteoporosis as a consequence of higher BMD at a younger age. Nearly all circulating IGFs are bound to IGF binding proteins (IGFBPs), which regulate IGF availability and prolong IGF circulation (61). The most common binding protein is IGFBP3 and acute resistance exercise has been shown to elevate IGFBP3 (68, 91).

Physical activity is one of the major non-pharmacological methods for increasing and maintaining BMD and bone strength. However, not all exercise is effective, so a prescription in terms of optimal type, intensity, frequency and duration is required. The 2004 Surgeon General's report on osteoporosis specifically recommends Tai Chi as a good exercise for fall prevention (39). A long-term randomized intervention study (116) concluded that Tai Chi is beneficial for increasing bone formation in the elderly. Although Yoga exercise has potential as an alternative way to increase BMD, the underlying mechanisms and scientific evidence are currently lacking for this type of intervention.

This literature review will cover bone physiology including the biochemical markers of bone turnover and major hormones for bone metabolism. In terms of bone

adaptation, mechanical loading will be reviewed including results from animal models. In addition, I will discuss recent intervention and novel exercise studies in relation to bone health.

Bone Physiology

Bone is the substance that forms the skeleton of the body. Embedded in the matrix are osteoblasts, osteoclasts, and osteocytes. Osteoblasts are bone cells that cause the deposition of bone tissue, bone-forming cells. They are derived from mesenchymal precursor cells in marrow that have the potential to differentiate into fat cells, chondrocytes or muscle cells (20). Osteoclasts are large, multinucleated bone cells that cause the resorption of bone tissue, bone-destroying cells. They are derived from hematopoietic precursor cells. Two cytokines are essential and sufficient for basal osteoclastogenesis, the first being receptor activator of nuclear factor kappa-B ligand (RANKL) and the second being macrophage colony stimulating factor (M-CSF) (126). For osteoclastic bone resorption, the osteoclast adheres to bone through the integrin $\alpha v\beta 3$, creating a sealing zone and releasing hydrogen ions (HCO^{3-} and H^{+}) through the ruffled border and dissolving the mineralized bone matrix into calcium and phosphate (Ca^{2+} and H_3PO_4) (106). Osteocytes, star-shaped cells, are the most abundant cells found in compact bone. Like osteoblasts, osteocytes develop from mesenchymal cells. Osteocytes are networked to each other via long cytoplasmic extensions that occupy tiny canals called canaliculi, which are used for exchange of nutrients and wastes. These cells are regularly dispersed throughout the mineralized matrix, connected to each other and to cells on the bone surface through dendritic processes generally radiating toward the bone surface and the blood supply. Osteocytes are thought to function as a network of sensory cells mediating the effects of mechanical loading through this extensive lacuna-canlicular network. Not only do

these cells communicate with each other and with cells on the bone surface, but their dendritic processes extend past the bone surface into the bone marrow. Osteocytes have long been thought to respond to mechanical strain by sending signals of resorption or formation, and evidence is accumulating to show that this is a major function of these cells (106).

There are two types of bone tissue: cortical and trabecular bone. Cortical bone, also called compact, or lamellar bone, is densely packed and makes up the majority of the skeleton (around 80%). Trabecular bone, also called spongy or cancellous bone, is more porous and surrounded by cortical bone.

Individual bones are composed of both types of bone tissue, but the relative proportion of trabecular and cortical bone varies (96). Cortical bone is composed of osteons, which are the functional units of bone. Osteons are organized into concentric layers of matrix called lamellae, which are surrounded by widely dispersed cells. The matrix is the intercellular space, and it is made up of organic and inorganic substances. Trabecular bone has the same cells and matrix elements as cortical bone, but it has a greater degree of porosity (96). About 80-90% of the volume of cortical bone is calcified; only 15-25% of trabecular bone is calcified (the remaining volume is occupied by bone marrow, blood vessels, and connective tissue) (9). Therefore, cortical bone is best suited for structural support and protection, and trabecular bone is best suited for bone's physiological functions. Owing to its large surface area, trabecular bone is able to remodel more rapidly than cortical bone. It is also in the trabecular bone that the greatest age-related loss in BMD occurs. Therefore, most osteoporotic fractures occur in areas composed predominantly of trabecular bone (wrist, hip, and spine) (96).

As in most processes in physiology, bone formation is extremely dynamic. Approximately 100% of the infant skeleton and 10-30% of the adult skeleton is replaced each year (18). This process of breakdown and buildup of bone is called remodeling. Old bone is resorbed by osteoclasts and new matrix is deposited by osteoblasts. The process allows precise control of calcium and phosphate metabolism and helps maintain healthy bone. It also enables bones to adapt to stress: bone forms when subjected to increased loads and is resorbed when stresses decrease. As a result of bone remodeling, then, bone mass may increase, stay the same, or decrease. Bone mass tends to increase during growth and decrease in old age (18).

Bone remodeling reflects the interrelationship between the structural and physiological functions of bone. Calcium not only is necessary to provide structural integrity of bone, but also is essential to the proper functioning of the heart, skeletal muscles, and nervous tissue. Only about 1 g of calcium (less than 1%) is present in the entire extracellular fluid of the body, compared to approximately 1150 g of calcium present in bone tissue. Blood calcium levels are normally maintained within the range of 9-11 mg/dL of blood (7, 84).

The primary hormones involved in regulating blood calcium levels and bone remodeling are parathyroid hormone (PTH), calcitonin, and calcitriol. Excess calcium in the blood leads to the release of calcitonin, which causes deposition of calcium in bone (84). Conversely, when blood calcium levels drop below normal (about 10 mg/dL), PTH stimulates osteoclast activity, causing calcium to be released from the bone. This release of calcium from the bone causes blood calcium levels to increase and BMD to decrease (84). Calcitrol is important for the absorption of calcium from the intestines. It regulates calcium and phosphorus levels in the blood by promoting

their absorption from food in the intestines, and by promoting re-absorption of calcium in the kidneys (44).

Other hormones that play an important role in skeletal health are the sex steroids (estrogen and testosterone) and GH. These hormones stimulate the protein formation necessary for bone growth and are responsible for the closure of the epiphyseal plate, which will ultimately determine bone length and thus a person's height (7). Estrogen is important in promoting calcium retention and acts as an inhibitor of PTH. The loss of the protective role of estrogen on the skeletal system has important consequences for women after menopause or during secondary amenorrhea. A decrease in estrogen has the net result of increasing bone resorption. Hormones are themselves stimulated by other factors, including physical activity (96).

Mechanical Loading

In response to the mechanical forces exerted on the bones, the bone adapts to altered physical stimuli, termed bone adaptation and it requires bone cells to detect mechanical signals and integrate these signals into appropriate changes in the bone architecture (132). In 1892, Wolff (147) proposed that bone architecture is determined by mathematical laws: the thickness and number of trabeculae (i.e., the distribution of mass) must correspond to the quantitative distribution of mechanical stresses, and the trabeculae must be stressed axially in compression or tension. This principle is demonstrated by Pauwels' studies (94) showing the correspondence between stress trajectories and trabecular architecture in the proximal femur. In 1998, Turner (132) introduced three major rules for bone adaptation to mechanical stimuli: 1) bone adaptation is driven by dynamic, rather than static, loading; 2) the mechanical loading period need only to be short in duration and extended durations result in diminishing

returns; and 3) bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals.

Mechanical loading of the skeleton via exercise has been shown to improve bone mass in human (16, 113) and animal models (133, 140). The size, shape, and strength of the bone are regulated in part by the mechanical forces applied to bone during daily physical activities. These forces are created during movement by muscle contractions and by impact with external objects such as the ground in walking or a ball in tennis. Bending, compression, tension, torque, and shear forces all cause bone deformation, which is quantified as strain (change in length/original length). The complex movement patterns associated with exercise result in complex strain patterns that vary in magnitude, rate, and frequency throughout the bone (27).

Mechanical stimuli are considered to be anabolic to bone. High magnitude and low frequency impacts such as from running have been recognized to increase bone and muscle mass (34, 137). However, the opposite stimulus, a low magnitude and high frequency (LMHF) mechanical load experienced in activities as low impact as when standing, has also been shown to be anabolic to bone (109). Recently, an LMHF mechanical loading device has been developed to treat bone loss by applying a low magnitude mechanical signal at a high frequency to the whole animal or human. The LMHF mechanical load has been shown to be effective in treating musculoskeletal pathologies in a number of subjects during research and clinical trials, including in animals (110), young women with low bone mass (41), and postmenopausal osteoporotic women (108).

Previous studies have indicated that the LMHF mechanical loading has been shown to normalize and prevent bone loss in both animals and humans (41, 108, 110). However, the underlying molecular changes and the target cells by which LMHF

mechanical loading alleviate bone loss are not known. Patel et al. (93) studied whether direct application of LMHF mechanical loading to osteoblasts alters their cell responses, preventing decreased bone formation induced by disuse or microgravity conditions. Preosteoblast 2T3 cells were exposed to a disuse condition using the random positioning machine (RPM) and intervened with an LMHF mechanical load (0.1-0.4 g at 30 Hz for 10-60 min/day). Exposure of 2T3 cells to the RPM decreased bone formation responses as determined by alkaline phosphatase (ALP) activity and mineralization even in the presence of a submaximal dose of one morphogenic protein 4 (BMP4) (20 ng/ml). However, LMHF mechanical loading prevented the RPM-induced decrease in ALP activity and mineralization. Mineralization induced by LMHF mechanical loading was enhanced by treatment with BMP4 and blocked by the BMP antagonist noggin, suggesting a role for BPMs in this response. In addition, LMHF mechanical loading rescued the RPM-induced decrease in gene expression of ALP, runx2, osteomodulin, PTH receptor 1, and osteoglycin. This study suggested that low impact loading in animals and humans may directly stimulate osteoblasts and subsequent bone formation responses and also provided insight into the potential cellular and molecular changes regulating how such low level mechanical loads could prevent or normalize bone loss.

During physiological load-bearing, tibiae and ulnae get loading from compression, and undergo bending such that the periosteal surface have both bending and compression components along with some shear and torsion. Bone adaptation to mechanical stimuli at tibial and ulnar sites may differ (71). Kuruvilla et al. (71) investigated whether the bone adaptation response is site-specific and whether it is dependent on the animal's strain. They used two bone sites, the right tibia and right ulna, in three mouse strain: 1) C57BL/6J (B6), 2) DBA/2J (D2) and 3) C3H/HeJ (C3).

Forty-five adult female mice from these three inbred strains (B6, D2, and C3) were loaded at the right tibia and ulna *in vivo* with non-invasive loading devices. Each loading session consisted of 99 cycles at a force range that induced ~ 2000 microstrain at the mid-shaft of the tibia (2.5 to 3.5 N force) and ulna (1.5 to 2 N force). The right and left ulnae and tibiae were collected and processed histologically to produce undecalcified cortical bone slides. Standard histomorphometry techniques were then used to quantify new bone formation. The histomorphometric variables included percentage mineralizing surface (%MS), mineral apposition rate (MAR), and bone formation rate (BFR). Net loading response (right-left limb) was compared between different strains at tibial and ulnar sites. Significant site differences in bone adaptation response were present within each strain ($p < 0.005$). In all three mouse strains, the tibiae showed greater percentage MS, MAR, and BFR than did the ulna at similar *in vivo* load or mechanical stimulus (strain). These data suggested that the bone formation due to loading is greater in the tibiae than the ulnae. Although, no significant stain-related differences were found in response to loading, the data showed greater trends in tibial bone response in B6 mice as compared to D2 and C3 mice. As a result, these data indicated that there are site-specific skeletal differences in bone adaptation response to similar mechanical stimulus.

While the mechanical loading and disuse responses affect the adaptive response to bone strain, many other factors influence bone adaptation. Males and females respond differently to loading, and alterations in environment, genetic constitution, age, nutrition and other systemic biochemical influences have effects on loading responses (122). In girls, growth is slower than in boys, but bone growth and accrual is disproportionately greater when expressed in relation to muscle mass (112). The implication of this is that the effect of circulating reproductive hormones is to

increase the sensitivity of female skeletons to load compared to males. At the menopause, the reduction in circulating reproductive hormones has the opposite effect, so that the female skeleton is perceived as over-engineered and excessively massive. In this manner, bone loss after the menopause is a disuse phenomenon, brought about by the alteration in sensitivity of bone cells to loading in females by estrogen.

Biochemical Markers of Bone Turnover

The most widely used measurement of BMD cannot provide a representative picture of the skeletal imbalance between bone formation and resorption. Therefore, a very precise screening test with the ability to identify minor bone changes is needed. Bone turnover markers (BTMs) reflect whole body rates of bone resorption and bone formation. Specific assays for bone turnover have been developed that have aided in the diagnosis and management of metabolic bone diseases, including osteoporosis (35). BTMs are now widely used in clinical trials as measures of antiresorptive and anabolic effects (17, 129).

In intervention studies, resistance (33) and impact training (117) have been shown to enhance bone formation, but controversial results have also been reported with different types of training regimens (64, 89). Shibata et al. (117) investigated the influences of long-term walking training and walking and jumping training on BMD in premenopausal women (age mean ± SE, 35 ± 2). The subjects were randomly divided into two groups: a walking group (WG; n = 26) and a walking and jumping group (WJG; n = 15). The subjects were given instruction with a final goal of walking 10,000 steps per day in both groups for one year. Members of the WJG were instructed to jump straight up as high as they could, and were asked to jump and land with both legs, 10 times total each day. BMD was measured in the lumbar spine and proximal femur using DXA. Also, bone formation markers (Bone ALP, OC) and

resorption marker (NTX) were also measured. Despite the significant decrease in body weight ($p < 0.05$), no corresponding decrease in BMD was neither observed nor any significant difference in bone markers (OC, PTH, NTX). Bone ALP was significantly increased ($p < 0.05$) after one year, and the rate of this increase was greater in the WJG than in the WG group.

To determine the effects of moderate physical activity on BTMs in premenopausal women, Adami et al. (2) reported the correlation between simple physical activity and bone mass or BTMs and the changes in BTMs as a consequence of a few weeks of group gymnastics. This cross-sectional study involved 530 premenopausal women recruited from 20 different centers. The subjects were all healthy and were not on the contraceptive pill or other drugs known to influence bone metabolism. The women also were required to have regular monthly cyclic menses (cycles occurring every 25-35 days). The questionnaire administered included the time spent exercising (for example, walking outdoors, but also practicing any weight-bearing sport activities), which was ranked as 0, ≤ 30, or > 30 min/day. Blood samples were collected for BTMs: CTX, OC, N-terminal propeptide of type I procollagen (PINP). BMD at the lumbar spine and femoral neck were assessed by DXA. The bone formation markers (OC, PINP), but not the bone resorption marker CTX, were found to be significantly associated with the level of physical activity, and this association remained significant after adjusting the data by age and Body Mass Index (BMI). Mean spine and hip BMD values were positively associated with physical activity but this was statistically significant ($p = 0.05$) only for adjusted values of spine BMD.

The same researchers conducted an intervention study (2). Twenty-four healthy sedentary premenopausal women between the ages of 39 and 45 years

participated in this study and they were sedentary during the previous 6 months (walking <30min/day). Exercise training sessions were conducted as a group 3 or 4 days each week. Each session lasted ~90 min and the first 15 min were dedicated to walking at increased speed, and the remainder of the time to endurance exercises: running, walking on a treadmill, step-ups and stair climbing. The intensity of exercise was gradually increased over the 4 weeks of the program. The control group consisted of women who participated in the cross-sectional study, age-matched with the active group. Serum OC and PINP rose significantly, by 25%, in the active group. No changes were observed in CTX levels. The researcher concluded that both the cross-sectional and the longitudinal parts of these studies demonstrated that even minor changes in physical activity are associated with a clear effect on bone formation markers.

BTM levels can also be influenced by diurnal and seasonal factors, diet, menopausal status, and phase of the menstrual cycle. BTMs have been shown to exhibit cyclic variations during the menstrual cycle (24, 45, 152). These fluctuations appear to be related to cyclic variations in estradiol and progesterone. Gass et al. (37) investigated the further information on the physiologic pattern of BTM variation associated with the menstrual cycle in healthy premenopausal women in the United States. This study included 58 premenopausal women aged 18 to 35 years with a 3-month history of normal menstrual cycles. The women underwent blood and urine sampling to determine BTM levels during on menstrual cycle and up to day 3 of the next menstrual cycle. Samples were taken after an overnight fast of 6 or more hours. Blood was drawn at the same time each day, between 8 and 10 am to limit the effect of circadian fluctuations of biochemical bone markers. Mean CTX values were 0.48 ng/mL during the follicular phase, 0.47 ng/mL at serum luteinizing hormone peak,

and 0.43 ng/mL during the luteal phase. Additionally, the mean percent change from luteinizing hormone peak varied from +4.35% during the follicular phase to -5.11% during the luteal phase ($p = 0.0014$). Mean CTX levels during the early and through mid follicular phase were significantly higher than levels during the mid and late luteal phase. The pattern for NTX was similar to that of CTX but not statistically significant. There was a statistically significant tendency for PINP levels to be lower during the follicular phase relative to the luteal phase. Levels of OC and Bone ALP did not vary significantly during the menstrual cycle. The authors concluded that levels of some bone turnover markers varied during the menstrual cycle. A statistically significant change in CTX (9.46%) occurred between the follicular phase and luteal phase of the menstrual cycle.

Hormone Response and Bone Metabolism

Bone remodeling is regulated by systemic hormones and IGF-I has received much attention because they are involved in longitudinal bone growth prenatally and during puberty (57, 107), and age-related decreases in both systemic and bone tissue levels have been documented (12, 90). Approximately 99% of circulating IGF-I is bound to six specific high-affinity IGF-binding proteins that are produced in osteoblasts and other cell types and modulate IGF action in a positive or negative manner (60). A major portion of IGF-I is bound to IGFBP3, which is a quantitatively predominant IGFBP in the circulation and is considered to be positively regulated by GH and/or IGF-I (13, 26). Previous studies have examined the relationship between hormone concentrations and bone loss and how exercises affect hormone responses mostly in postmenopausal women.

Liu et al. (78) examined IGF-I, osteoprotegerin (OPG), leptin, osteocalcin (OC), and urinary excretion of N-terminal telopeptide of type I collagen (NTX) to

determine which one demonstrates the greatest utility in the early detection of women with low bone mass or osteoporosis in pre- and postmenopausal women. Two hundred eight-two premenopausal and 222 postmenopausal women ages 20-75 years were investigated by measurement of BMDs at lumbar spine and femoral neck using DXA, together with serum concentrations of IGF-I, OPG, leptin, OC, and urinary NTX. It was revealed that serum levels of IGF-I and leptin changed the earliest, with both markers significantly decreasing ($p < 0.0001$) or increasing ($p = 0.02$), respectively, at age 30. However, IGF-I was the only early parameter that had the capacity to differentiate the low bone mass/osteoporosis women from the normal women ($p < 0.0001$). In the premenopausal women subgroup analysis, the low bone mass women (30/282, 10.6%) were older (38.2±1.7 vs. 34.5±0.5 years), with lower serum levels of IGF-I (215.1±22.4 vs. 278.8±9.4 ng/ml; $p = 0.02$) and less lean mass (33.1±0.6 vs. 34.8±0.2 kg; $p = 0.010$) than women with normal bone mass. After controlling for age, the serum level of IGF-I was weakly, but still significantly, positively correlated with lean mass ($r = 0.17$, $p < 0.001$). It is well known that both BMD and serum concentrations of IGF-I decrease with age. However, it was clearly demonstrated in this study that a decrease in serum IGF-I level occurs almost 20 years before a significant reduction of BMD, thus making it a potential marker for subsequent bone loss. Furthermore, they found that 10.6% of premenopausal women had already developed osteopenia, with a mean age of 38, and that their serum levels of IGF-I were also reduced. The researchers concluded that measurement of serum IGF-I in young women may help in the early identification of those at risk for developing low bone mass and osteoporosis.

There are limited studies that report higher IGF-I in physically active versus nonactive individuals, but the type of activity (aerobic vs. muscle building) associated

with higher IGF-I differs (28, 63). Davee et al. (28) reported that performing muscle-building exercise was associated with higher IGF-I levels than was engaging in aerobic exercise. However, Kelly et al. (63) demonstrated a strong relationship between aerobic capacity and IGF-I in mature pre- and postmenopausal women, but no independent relationship between muscle strength and IGF-I. To gain a better understanding of the relationship between IGF-I and muscle and bone, Snow et al. (123) examined the relationships between IGF-I and IGFBP3, body composition, and BMD in collegiate runners (n=13), gymnasts (n=10), and noncompetitive women (n=10). They assumed that the ratio of IGF-I to IGFBP3 may be an important marker of IGF-I bioavailability because IGFBP3 is fully saturated with IGF-I and II, a higher ratio could mean that there is more IGF-I than IGFBP3, allowing IGF-I to more easily be transported into tissues by lower molecular weight binding proteins. The researchers found that gymnasts have higher IGF-I and IGF-I/IGFBP3 than runners and higher IGF-I/IGFBP3 than healthy control women. BMD was greater in gymnasts than in runners at the spine and hip and higher than controls at the hip; lean mass was greater in gymnasts than runners. Significant positive correlations were observed between IGF-I and IGF-I bioavailability and bone and lean mass. Both lean mass and IGF-I bioavailability predicted femoral neck BMD. Thus, these results confirmed that IGF-I was higher in gymnasts who have greater BMD and lean mass than runners and that IGF-I would independently predict lean and bone mass.

Exercise Interventions and Bone in Premenopausal Women

Peak bone mass in women is attained between late adolescence and the third decade of life (150) and is thought to be a predictor of osteoporosis risk in postmenopausal years (138). Besides genetic and hormonal factors, modifiable factors contributing to peak bone mass include calcium intake and physical activity and these

are determinants of risk for osteoporosis (10, 11, 67, 138). Cross-sectional studies in premenopausal women consistently showed higher BMD in women who perform weight-bearing or strength-training exercise compared to normally active controls (28, 62). However, previous intervention studies have shown inconsistent effects of exercise training on BMD (23, 31, 42, 79, 120).

Singh et al. (120) assessed the effect of 9 months of strength training on total body and regional BMD in 58 premenopausal women aged 30-50 years. Participants were randomized to either twice weekly 50-min supervised strength training for 15 weeks followed by 24 weeks of unsupervised training (treatment group) or a control group. At each of these sessions, participants performed three sets each of 9 common strength-training exercises (squats, leg press, leg extension, seated leg curl, lat pulldowns), with as much weight as they could lift for 8-10 repetitions. DXA scans were taken and analyzed for body composition (lean and fat mass) and BMD was measured for total body and its sub-regions (spine, hip, arms and legs). All measurements were performed at baseline, 15, and 39 weeks. Analysis of covariance was used to assess group differences in BMD change adjusted for baseline BMD, weight, energy, and calcium intake. BMD increased at all sites in the exercise group except for a slight decrease in the hip at 39 weeks. In particular, spine BMD increased significantly by 2.2% in the intervention group ($p < 0.05$) and did not change in the control group. However, the between-group differences for change in BMD were not statistically significant at any site. This study added to previous work by exploring the effects on BMD of a strength-training only program in premenopausal women with a broad range of BMI (19-36 kg/m^2) and body fat percentage (23-54%) in a randomized controlled trial. These results showed no significant treatment effect of strength training on total body or regional BMD. Although group differences in change were

not significant, the intervention group had a significant increase in spine BMD (2.2%, $p < 0.05$), while the control group had no change. Similar to this study, previous strength-training intervention studies have found higher but insignificant increases in regional BMD (23, 42). In contrast, other prospective studies showed either a significant increase (31, 79) or a significant decrease in BMD (87) at various skeletal sites in the strength-training group. In agreement with previous studies (15, 86), it may be difficult to observe a significant BMD increase in premenopausal years after peak bone mass has been achieved.

Based on published guidelines by the U.S. Department of Health and Human Services (114), Warren et al. (141) investigated the effect on proximal femur and lumbar spine BMD and aBMD of twice-weekly strength training in premenopausal women compared to a standard care control group. One hundred and forty-eight overweight, sedentary, premenopausal women aged 25-44 were randomized to progressive strength training (ST, n=72) or standard care (CO, n=76) for 2 years. The exercise program consisted of twice-weekly strength training of three sets of 8 to 10 repetitions using variable resistance machines and free weights that stressed all major muscle groups following published guidelines (114). After the initial 3 weeks, the intensity of the strength training was set so that the participants were able to complete three sets of 10 repetitions. When each participant was able to complete four sessions of three sets of 10 repetitions, the weight was increased by the smallest increment possible. This progression continued throughout the first year. During the second year of the intervention, participants were allowed to maintain the highest weight lifted for each exercise, although some continued to increase. Measurements occurred at baseline, 1 yr, and 2 yr. Proximal femur and lumbar spine BMD and aBMD were measured by DXA. aBMD showed little change and did not differ between groups at

any site. Femoral neck BMC showed a significant difference in the slopes between ST and CO ($p = 0.04$) with no change in the ST group and a 1.5% decrease in the CO group. There were no significant between-group differences at any other measurement site. These results in aBMD differ from previously published strength training trials in premenopausal women with an intervention of similar length (31, 79). In an 18-month study of 56 premenopausal women comparing thrice weekly strength training to a non exercising control group, Lohman et al. (79) reported a significant aBMD increase of 2.3% in the lumbar spine and 1.8% in the femoral trochanter and a significant increase of 1.4% in the femoral neck in the strength training group relative to changes in the control group. In contrast, Warren et al. (141) showed non significant aBMD changes of 1.2% and 0.8% in the trochanter and lumbar spine, respectively, with no change in the femoral neck or total femur. They assumed that the lower frequency of strength training in this study (141) compared to the previous trials (31, 79)may have accounted for the differences seen.

Most exercise interventions aimed at improving bone health in women have been general rather than specific to either the hip or spine. The limited exercise programs specific to the hip or spine have shown that impact (jumping) training increased hip, but not spine BMD (10, 11) and that a 3-year back-strength training program did not increase spine BMD (119). Winters-Stone et al. (144) studied the response of bone at specific skeletal sites to either lower body exercise alone or complemented with upper body exercise in premenopausal women. Thirty-five exercisers and 24 age-matched controls completed the 12-month study. Exercising women (n=35) were randomly assigned to either lower body resistance plus jump exercise (LJ, n=19) or to lower and upper body resistance plus jump exercise (LUJ, n=16). Exercise participants were asked to attend 3 exercise sessions per week with at

least 1 day of rest between sessions. Women in both training groups performed lower body exercise. The lower body training program consisted of 9 sets of 10-12 jumps and 9 sets of 10-12 repetitions of lower body resistance exercises. Jumping routines varied in type and height, but totaled 100 for each session. Lower body exercises (squats, lunges, and calf raises) were performed immediately following jumps. Each session consisted of 3 sets of 10-12 squats, 6 sets of 10-12 lunges, and 2 sets of calf raises. Jump and resistance intensity were progressively increased using weighted vests and calculated as a percentage of body weight (%BW) over the program to a final intensity of 10% BW for jumps and 13% BW for resistance training. For upper body exercise, following jumps, participants randomized to LUJ group performed 3 sets of 8-12 repetitions of upper body exercises at 8-12 RM. Exercises consisted of the following: upright row, one-arm row, latissimus dorsi pull-down, chest press, chest fly, biceps curl and triceps extension. BMD at the total hip, greater trochanter, femoral neck, lumbar spine and whole body were measured by DXA at baseline, 6 and 12 months. The results showed that significant differences in greater trochanter BMD between each exercise group and controls, but not between exercise groups (2.7±2.5% and 2.2±2.8% vs. 0.7±1.7%, for LJ and LUJ vs. controls, respectively; $p < 0.02$) and near significant group differences at the spine ($p = 0.06$). No significant differences among groups were found for femoral neck, total hip or whole body BMD. These data supported the site-specific response of spine and hip bone density to upper and lower body exercise training, respectively.

A recent meta-analysis of selected randomized controlled trials (RCT) that have investigated the effect of exercise on bone mass has revealed positive effects of exercise on the lumbar spine and femoral neck in premenopausal women (138). Although collectively RCT showed that regular exercise could delay or reverse bone

loss in women (138, 146), the exercise-induced increases in BMD reported in longitudinal studies are much smaller and less convincing than the results of cross-sectional studies (28, 62). As described earlier, the lack of consistency between studies is probably caused by differences in the study populations (pre- or post-menopausal women), the type, length and intensity of the exercise programs and the duration of the follow-up periods. There are a number of limitations to some of the earlier RCT concerning exercise and bone: 1) the exercise programs were sometimes too general rather than specific in loading the hip or spine, which were the clinically-important sites measured; 2) considering the physiological limits of bone formation and remodeling, the duration of the protocols was sometimes too short to observe significant effects of a lifestyle intervention like exercise; 3) most studies used BMD as their primary outcome measure, which is a suboptimal surrogate for bone fracture rates; 4) many trials had small sample sizes that are subject to large type II error (6). Intervention studies that have investigated the effects of exercise training on BMD cover a range of exercise types, intensities, frequencies and durations, but very few have compared different prescriptions within the same trial, so it is difficult to determine the training program. This aspect is important because as well as recommending certain types of exercise and for how long to exercise, individuals also need to know how many times per week to exercise. It is clear that there is a need for randomized trials that compare the effects of different exercise prescriptions on bone density.

Novel Exercise Interventions

Evidence regarding osteogenic effects of exercise in pre- and postmenopausal women largely comes from studies involving high-impact exercise or power training, such as jumping or weightlifting (67, 144). Recently, different modes of exercise have

been investigated for their ability to provide beneficial effects on BMD. There have been few attempts to investigate alternative activities for bone health.

Wayne et al. (142) examined the use of Tai Chi as a potential intervention for postmenopausal women with low BMD. They reported that a number of characteristics of Tai Chi practice maybe effective for maintaining bone density and improving postural control as described in recent reviews (76, 131, 145). These characteristics and their purported effects include: 1) a constant shifting of weight from one leg to the other, which facilitates improved lower-extremity strength and /or mechanical load and dynamic standing balance. 2) an emphasis on maintaining a vertical posture with an extended head and trunk position, which promotes a less flexed posture. 3) the use of different parts of the body taking turns playing the role of stabilizer and mover, which enables movements to be executed smoothly without compromising balance and stability. 4) a continuous, slow, even tempos that facilitates sensory awareness of the speed, force, trajectory, and execution of movements, as well as awareness of the external environment. 5) the symmetrical and diagonal arm movements of Tai Chi and increase trunk rotation around the waist. 6) moderate knee flexion, which lowers the body's center of gravity and 7) flowing circular and spiraling movements, which promote joint flexibility.

Previous authors assumed that such changes may translate into increased mechanical load on key regions of the skeleton including the femur, hip, and lower spine (76, 131, 145). Wayne and colleagues (142) summarized 6 eligible studies. In this systematic review, 2 studies were randomized controlled trials (RCTs) (21, 151), 2 were nonrandomized prospective parallel cohort studies (97, 148), and 2 were static cross-sectional comparisons (43, 98). Results across the 6 studies suggest that long-term postmenopausal Tai Chi practitioners have higher BMD than age-matched

sedentary controls, and have slower rates of bone loss. In one cross-sectional study of postmenopausal women, Qin et al. (98) used DXA to compare BMD of 48 long-term Tai Chi practitioners with 51 age-matched sedentary controls. Subjects in the Tai Chi group had significantly higher BMD in the lumbar spine (7.1%), the greater trochanter (7.2%), and Ward's area (7.1%) of the proximal femur ($p < 0.05$). Similar magnitudes of BMD differences between Tai Chi and age-matched sedentary controls were observed in an earlier study conducted by the same research group in a similar population (97). This earlier study also tracked changes in BMD over a 12-month period and found that rates of both trabecular and cortical BMD loss in the distal tibia were approximately 50% lower in the Tai Chi group. One methodologically sound RCT of postmenopausal women observed that those randomized to 12 months of regular Tai Chi training (n=67) exhibited 3.6-fold (trabecular) to 2.3-fold (cortical) reductions in rates of BMD decline in the distal tibia as measured with pQCT ($p < 0.05$), as compared to a no-exercise control group (n=65). No significant differences between groups were reported for BMD of the spine or femur as measured with DXA (21). In addition, a nonrandomized cross-sectional study (148) provided qualitative data suggesting that Tai Chi improves perimenopausal symptoms including hot flashes and abdominal distention. Overall, Tai Chi appears to be safe for peri- and postmenopausal women. No significant adverse effects were reported in any of the 6 studies evaluated.

Shen et al. (116) compared the effects of Tai Chi and resistance training on bone metabolism in the elderly. Twenty-eight sedentary subjects (22 women and 6 men) were randomized into either Tai Chi (n=14) or resistance training (n=14) groups that participated in 40 min of exercise per session, 3 sessions per week for 24 weeks. The resistance training consisted of one set of 10-12 repetitions for bench press, leg

press, leg curl, leg extension, and seated row on a resistance exercise machine, as well as shoulder press and arm curl exercises using dumbbells. Subjects in the resistance group rested for about one minute between exercises and the exercise intensity was set at 50% of 1RM. Subjects in the Tai Chi group were taught by an experienced instructor and practiced the 24-form simplified Yang-style, the most popular form of Tai Chi among dozens currently being practiced worldwide (139). In addition to the 5 min of warm-up and 5 min of cool-down exercise, the routine 24-form simplified Yang-style Tai Chi was repeated 5 times during the 30 min training period based on the standard speed of about 6 min per routine. The outcome measures assessed were the concentrations of serum Bone ALP, pyridinoline (PYD), PTH, and calcium, and urinary calcium. After 6 weeks: 1) both Tai Chi and resistance training resulted in higher levels of serum Bone ALP relative to baseline and the Tai Chi group exhibited a greater increase in serum Bone ALP than the resistance training group, 2) there was an increase of serum PTD in the resistance training group only, not in the Tai Chi group, and 3) the Bone ALP/PYD ratio was higher than baseline only in the Tai Chi group, and the increase of the ratio in the Tai Chi group was greater than that in the resistance training group. After 12 weeks, the increase in serum PTH in the Tai Chi group was greater than that in the resistance training group. After 24 weeks, there was a reduction in urinary calcium level in the Tai Chi group relative to the baseline. The researchers concluded that these findings supported Tai Chi as being beneficial for increasing bone formation in the elderly, and that long-term application is needed to substantiate the effect of Tai Chi as an alternative exercise in promotion of bone health.

Yoga is a popular form of activity that incorporates various body postures and poses. Most people believe that Yoga has beneficial effects on BMD, however, there

is little scientific evidence to substantiate this belief. Sweesy- Barger (127) compared measures of BMD in a group of postmenopausal women who regularly participated in Yoga for a minimum of 3 years, at least twice a week in sessions lasting 60 minutes or longer (Y, n=31) to a group of age-matched non-Yoga participants performing less than 2 hours of physical activity per week over the past 3 years (NY, n=31). The Yoga group had significantly lower mean body mass, percent body fat, fat mass, and BMI compared to the inactive group. Mean body mass was 65 kg in the Yoga group and 73 kg in the control group. The mean percent of body fat was 34% in the Yoga group and 42% in the control group. This cross-sectional study showed no significant differences in BMD at the spine, femur, or whole body. Initial analysis indicated that wrist BMD was significantly higher in NY compared to Y. When covariates were included in the analysis, however, no significant difference in wrist BMD was observed. These results suggest that Yoga activity may not provide an adequate stimulus to produce positive effects on BMD.

A recent study by Phoosuwan et al. (95) investigated the effects of the weight- bearing Yoga training on bone markers in postmenopausal women. The subjects were divided into experimental (Y, n=19) and control (C, n=14) groups. The experimental group attended the 12-week weight-bearing Yoga training 3 days a week, 50 minutes a day while the control group maintained their normal lives. The Yoga training consisted of Tree, Downward Facing Dog, Warrior III, Triangle, and Half Moon poses. They measured bone resorption marker (β-CrossLaps) and bone formation marker (PINP) at baseline and after the 12-week Yoga training. The findings indicated that the mean scores on the bone resorption markers were significantly different (Y, -26.94% vs. C, -0.77%). This study concluded that weight-

bearing Yoga training had a positive effect on bone by slowing down bone resorption in postmenopausal women.

Summary

Numerous exercise intervention studies have reported that the mechanical load from exercise that generates relatively high ground-reaction and joint-reaction forces (resistance training and plyometrics) acts to increase BMD in femoral neck and/or lumbar spine in sedentary women (10, 11, 79). However, differences in BMD responses are observed depending on the type of exercise. Previous studies have indicated that weight-bearing exercises positively affect bone metabolism, and biochemical markers have been used to evaluate the mechanisms involved (17, 70, 83). High-intensity progressive resistance training was shown to be effective for increasing BMD at the lumbar spine but not the femoral neck in premenopausal women (85). Among non-traditional forms of exercise for bone health, Tai Chi (a traditional Chinese martial art) has been recommended for osteoporotic women as a safe and effective exercise for bone density maintenance. For Yoga, no studies to date have examined intervention Yoga training studies to investigate their effects on bone metabolism and hormone responses.

CHAPTER III

METHODOLOGY

The purpose of this study was to examine the effects of an 8 month Yoga exercise program on bone biomarkers (Bone ALP and TRAP5b), BMD of the total body, dual femur, lumbar spine, tibia, forearm and bone geometry characteristics in premenopausal women. Subjects were healthy premenopausal women, between the ages of 35 and 50 years, who were randomly assigned to either the Yoga intervention group or to the control group. A secondary purpose was to examine responses of IGF-I, body composition, and muscle strength (1RM) to the Yoga intervention.

Subjects

Thirty-four healthy premenopausal women between the ages of 35 and 50 years were recruited from the University of Oklahoma and the surrounding Oklahoma City metro via flyers posted in public areas, an advertisement in local newspapers and mailed to prospective subjects at the University of Oklahoma, Norman campus (Appendix A). The subjects had not been engaged in resistance training or in Yoga exercise for at least 12 months prior to the study. The subjects were not taking hormonal contraception and they self-reported having regular menstrual cycles. Each subject completed the informed consent form (Appendix B), health history, menstrual history, bone-specific physical activity, 3-day dietary log, and calcium intake questionnaires (Appendix C). All methods and procedures were approved by the University of Oklahoma Institutional Review Board (IRB No.12669) (Appendix D).

Inclusion Factors

1. Subjects were normal healthy premenopausal women volunteers between the ages of 35 and 50 years.

2. Subjects were free of chronic back or joint problems, cardiovascular disease, non-smokers, not pregnant, not on hormonal birth control, not taking antihypertensive drugs or any medication that affects bone density.
3. Subjects had not participated in a weight training program and Yoga exercise for at least 12 months prior to the study.
4. Subjects were medically stable, ambulatory, and capable of undergoing physical strength testing and training.

Exclusion Factors

1. Individuals who were outside of the 35-50 years age range and who exceeded the weight limit of the DXA (300 pounds).
2. Individuals who were taking medications known to affect BMD such as steroid hormones, calcitonin, or corticosteroids.
3. Individuals who did not have regular menstrual cycles, who had thyroid problems or who were diabetic.
4. Any persons with physical and mental disabilities preventing them from being strength tested and trained, including orthopedic or arthritic problems, were not allowed to participate.

Research Design

Once prospective subjects were screened by interviews and questionnaires, the subjects were randomly assigned either to a Yoga exercise group (YE, n=16) or a control group (CON, n=18). Sixty-four Yoga sessions were offered two times per week with one day between sessions for 8 months in morning sessions (6:30 - 7:30 am, Mondays/Wednesdays) at the Huston Huffman Center in the University of Oklahoma. Subjects in CON group did not receive the Yoga exercise intervention and

they were encouraged to maintain normal daily lifestyle monitored by the bone-specific physical activity questionnaire at 2 month intervals for 8 months.

On the first day of testing, all subjects visited the Bone Density Laboratory at the Department of Health and Exercise Science on the University of Oklahoma-Norman Campus to complete the informed consent form, calcium intake, 3-day dietary log, health history, menstrual history, and bone-specific physical activity questionnaires. After 8 months, subjects completed menstrual history, bone-specific physical activity, and calcium intake questionnaires. Baseline measurements included five DXA scans (total body, dual femur, lumbar spine, forearm), 5 pQCT scans (tibia 4%, 38%, 66%; forearm 4%, 38%), urine sample to test hydration status, and a fasting blood draw. On the second day, subjects completed strength (1RM) testing for upper body (lat pull down, shoulder press, biceps curl) and for lower body (leg press, knee extension, knee flexion) (Appendix E). DXA scans, pQCT scans, and 1RM testing were assessed at baseline and at 8 months. Blood draws were obtained in the morning following an 8-hour overnight fast at baseline and 2-3 days after the last Yoga session. For urine collection, subjects were asked to drink 1 L the night before the testing to be well hydrated. Subjects in the YE group were asked to maintain their normal daily activities, which should not include any resistance exercise for 8 months. The women in the CON group completed all testing measurements at baseline and 8 months without Yoga exercise sessions and were instructed to simply maintain their usual level of physical activity for the same time period. Figure 1 shows an overview of the recruitment process and research design.

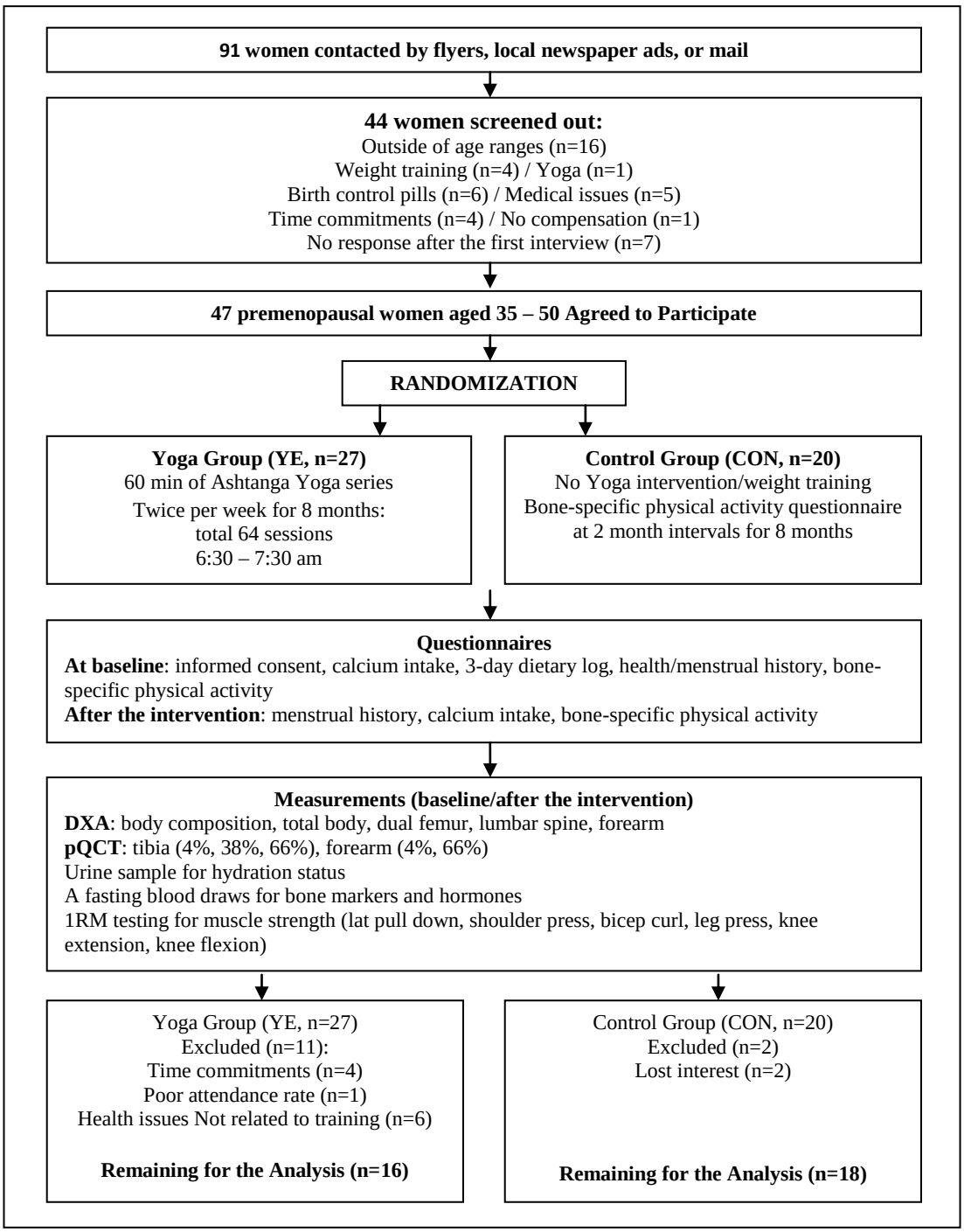


Figure 1. Overview of the recruitment process and research design.

Yoga Exercise Training

Yoga classes were offered on Mondays and Wednesdays each week, from 6:30 to 7:30 AM for 8 months. Subjects performed 60 minutes of an Ashtanga Yoga series, and session intensity was progressively increased. Each Yoga session consisted of 15 minutes of warm-up exercises, 35 minutes of asanas (Yoga postures) and 10

minutes of cool-down with relaxation (the corpse pose). During the first 5 minutes of warm-up, subjects were instructed to inhale and exhale through the nose, Ujjayi breathing, one breath for each movement, and they were encouraged to breathe using the Ujjayi breathing technique throughout the sessions. Dynamic and static stretching were introduced during the warm-up at the beginning with either sitting, supine, or standing postures. A certified Yoga instructor led all Yoga sessions and precisely taught Yoga postures with a consistent manner of instructions. Modified postures were taught to subjects who were not able to perform the standard postures. Yoga blocks and straps were provided for subjects who were willing to use them. Subjects were encouraged to perform all asanas as accurately as possible. Each posture was performed in both directions (right and left) and the Yoga session routines were performed in the same order. Thirty five minutes of asanas consisted of sun salutation (I, II), standing, balancing, sitting, supine postures, and core exercise (Appendix F). During the first 4 months, the sun salutation I with a triangle pose and warrior series were instructed and jumping was progressively included. Sun salutation II including jumping was performed during the last 4 months. The number of sun salutation I and II performed with jumping was progressively increased in each month. The asanas were static and held for approximately five to ten breaths each. Subjects recorded their heart rate (HR) using the palpation method for 15 seconds and the Rating of Perceived Exertion (RPE) at the beginning of the Yoga exercise, after sun salutations, and before corpse pose every Wednesdays (Appendix E). Subjects were asked to wear appropriate clothing (shorts or sweat pants) with at least one hour fasted status and were informed to know pre-considerations for Yoga exercise (Appendix G). The room was maintained at a temperature of 21-23 °C.

Body Composition Measurement

Dual Energy X-ray Absorptiometry (GE Lunar Prodigy, GE Medical Systems, encore 2002 Software, version 10.50.086, Madison WI) was used to measure body composition of the whole body. After having the subject remove footwear, the subject's height and weight were obtained using a wall stadiometer and a Tanita BWB-800 digital scale (Tanita Corporation of Ameroca, Inc., Arlington Heights, IL). All metal, plastic objects or other high density objects associated with the subject's clothes were removed. The subjects were asked to lie down on the DXA table in the supine position. The participant was centered on the table within 60 cm of the scanning area. The subject's shoulders and hips were centered, and the hands were placed by the side of the legs. Velcro straps were placed around the knees and ankles to hold feet together for the duration of the scan. A qualified technician performed all total scans at baseline and after an 8-month of Yoga exercise program. The coefficient of variation (CV %) for percent fat and bone free lean body mass (BFLBM) and total fat mass of DXA scans are 1.87%, 1.27%, and 1.62%, respectively.

For measuring hydration status, a refractometer (VEE GEE®, Model CLX-1) was used to measure urine specific gravity for hydration status. All subjects were encouraged to drink water the day before the testing to be well hydrated. If subjects had urine specific gravities out of range (1.004 – 1.029), they were not allowed to undergo the body composition assessment. If subjects failed the hydration test, they revisited until the urine specific gravity was within the range. Assessment of hydration status protocol was performed by the following procedures:

- Evaluator assistant wears latex gloves during the assessment.
- Subject is provided a cup (marked by identification number).

- Subject is then instructed to provide a 2-3 oz-urine sample (mid-stream) in the urinal/bathroom under supervision.
- Urine specific gravity is then measured by the refractometer.
- Open the daylight plate and apply one or two drops of the sample solution to the surface of the prism.
- Gently close the daylight plate over the prism. The sample solution should spread as a thin, even layer between the daylight plate and the prism.
- Looking through the eyepiece, hold the refractometer and direct the daylight plate upwards towards light.
- The reading is taken at the point where the boundary line of the blue and white fields crosses the scale.
- When each measurement is complete, the sample must be cleaned from the prism using tissue paper and water.

Bone Mineral Density Measurements

Dual Energy X-ray Absorptiometry (DXA):

Dual Energy X-ray Absorptiometry (GE Lunar Prodigy, GE Medical Systems, encore 2002 Software, version 10.50.086) was used to measure the BMD and BMC of total body; AP lumbar spine (L1-L4); dual proximal femur (femoral neck, trochanter, total hip); and the forearm (ultra distal radius 33%) sites assessed at baseline and the end of the 8 month study. The basic principle underlying DXA technology is that the attenuation of X-rays with high- and low-photon energies is measurable and is dependent on the thickness, density, and chemical composition of the underlying tissue. The attenuation, or weakening, of X-ray energies through fat, lean tissue, and bone varies due to differences in the densities and chemical composition of these tissues. These attenuation ratios at two different X-ray energies are though to be

constant for all individuals (68). The DXA was calibrated daily following the Quality Assurance (QA) and spine phantom calibrations procedures to monitor the machine's performance and to ensure no machine drift during the intervention period.

One qualified technician performed all scans and analyses using encore 2002 Software (GE Lunar Prodigy, GE Medical Systems, version 10.50.086). The standard scan mode was determined by the level of subject's umbilicus (Thick, >25 cm; Standard, 13-25 cm; and Thin, <13 cm). All scanning procedures were followed by the manufacturer's guidelines. For the lumbar spine measurement, the appropriate positioning block was chosen and placed under the subject's legs. The block orientation was recorded and used for all follow-up evaluations with that same subject. The positioning laser crosshairs was adjusted to approximately 5 cm below the umbilicus. The scan included at least part of L5, usually some of the iliac crest, and part of T12 with some rib. For the dual proximal femur, the subject's feet were secured to the DualFemur^{TM} positioner to maintain the appropriate internal rotation of the femur. The positioning laser was moved to a position 4 cm inferior to the greater trochanter or 1 cm inferior to the pubic symphysis in the midline of the thigh. The dual proximal femur scan included large and rounded, with soft tissue seen above the superior edge of the bone. For the ultra distal radius 33%, forearm scanning was performed with the subject comfortably seated in a standard-height chair that had no arms or wheels. The non-dominant arm was measured and the subject's elbow was flexed between 90° and 105°. The laser was positioned at a level that allowed visualization of the first row of carpal bones proximally. The ultra distal Regions of Interest (ROIs) excluded all cortical bone of the distal ulna and radius endplates so that the 33% ROI was fully visible. For scan analyses, all automatically analyzed scans were examined by the same technician and verified to be correct or adjusted

according to acceptable standard operating procedures. Example scans are given in Appendix H. The *in vivo* precision for the investigator on the DXA for the BMD of total body, spine (L1-L4), femoral neck (Right, Left), trochanter (Right, Left), total hip (Right, Left), and forearm are 0.68%, 0.76%, (1.36%, 1.38%), (1.39%, 1.30%), (0.77%, 0.96%) and 1.72%, respectively.

peripheral Quantitative Computed Tomography (pQCT):

Bone characteristics in tibia and forearm were determined by pQCT (XCT 3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) by a trained pQCT technician at baseline and at the end of the 8 month study. Scans were obtained on the non-dominant leg at 4%, 38%, and 66% and forearm at 4% and 66% of their lengths, respectively. For volumetric bone characteristics, total volumetric BMD (vBMD; mg/cm^3), trabecular vBMD (mg/cm^3), cortical vBMD (mg/cm^3), cortical thickness (mm), and Strength-Strain Index (SSI) (mm^3) were determined.

For the tibia scans (4%, 38% and 66%), the subject was asked to cross the non-dominant leg over the other leg and the technician measured the length of the tibia from the medial malleolus of the ankle to the tibia plateau of the proximal tibia. The subject was seated in the scanning chair with the non-dominant leg in the support straps and positioned in the center of the scanning area. The participant was asked not to move and to breathe normally during the scanning process. After the scout view was displayed, a reference line was set at the exact location of the proximal growth plate. After the end of the study, the same technician completed all scans in order to maintain consistency. The coefficient of variation (CV%) for 4% of the tibia total vBMD and trabecular vBMD are 0.83% and 0.57% , respectively. The CV% for 38% of the tibia vBMD, cortical vBMD, and SSI are 0.39%, 0.31% and 1.21 %,

respectively. The CV% for 66% of the tibia total vBMD, cortical vBMD, and SSI are 0.61%, 0.50% and 0.95%, respectively.

pQCT was performed on the non-dominant forearm by the trained technician at baseline and after the 8 month Yoga program. The forearm length was measured as the distance between the ulnar styloid and the olecranon. The subject's forearm was placed in the pQCT gantry and the non-dominant arm was secured with Velcro straps to prevent movement. Before the measurement, the subject was asked to search for a convenient and natural position to avoid movement during the scanning period. 4% and 66% of the forearm length were scanned. The CV % for 4 % of the forearm total vBMD, trabecular vBMD, and cortical thickness are 4.35%, 1.72%, and 5.60%, respectively. The CV% for 66 % of the forearm total vBMD, cortical vBMD, cortical thickness, and SSI are 1.28%, 0.63%, 1.21%, and 3.28 %, respectively. Example scans are given in Appendix H.

Muscular Strength Testing (1RM)

The subjects in the YE and CON groups performed one repetition maximum (1RM) testing to determine their upper and lower body muscle strength. A proper warm-up consisted of either walking or riding the stationary bicycle for 5 minutes. After familiarization with the resistance machines (Cybex Inc., Medway, MA), the subjects performed lat pull down, shoulder press, and biceps curl for upper body and leg press, knee extension, knee flexion isotonic resistance exercises for lower body at 8-10 repetitions of a light load (~ 50% of predicted 1RM) for warm-up. Following a 1 minute rest period, the load was increased until the subject was unable to lift the load through the full range of motion for a single repetition. 1RM was determined within 5 attempts. 1RM testing was supervised and recorded by trained staff (Appendix E).

Questionnaires

The subjects were asked to fill out questionnaires for bone-specific physical activity (BPAQ), health/menstrual history, calcium intake, and 3-day dietary log at baseline. After the 8 month intervention, menstrual history, calcium intake, and BPAQ were collected to compare with baseline values.

Bone-specific Physical Activity (BPAQ):

The bone-specific physical activity (BPAQ) assessment instrument was designed to be self-administered and to quickly and simply obtain a comprehensive account of lifetime physical activity (143). Based on type, frequency and years of physical activity, age-specific effects of mechanical loading on the skeleton are estimated to predict indices of bone strength at clinically relevant sites in both men and women. The BPAQ consists of independent sections for past (from one year of age) and current (previous 12 months). We used a total score from past and current physical activity to compare parameters of bone strength between two groups at baseline. The current physical activity score was used to monitor physical activity level for the CON group at 2 month intervals for 8 months.

3-day Dietary Log:

3-day dietary log was collected to determine whether their previous diet affects BMD values at baseline. Subjects were asked to record everything that they ate for two days during the week and one day during the weekend, including the food/drink item with brand names, the amount ingested, and method of preparation as specific as possible. The Diet Analysis Plus 9 database (Cengage learning, Inc., KY) was used for data analysis.

Blood Sampling

Resting blood samples (approximately 6 ml) were obtained by a phlebotomist in the morning following an 8 hour overnight fast at baseline and 2-3 days after the last Yoga session. Once the blood serum sample was collected from the antecubital vein, the sample was centrifuged to separate the serum from the red blood cells. The serum was aliquoted into microtubes labeled with subject ID, date, and tester's initial. It was stored in an -84° freezer housed in the Bone Density Laboratory until the bone marker and hormone assays were performed.

Bone Marker Assays

Bone Alkaline Phosphatase (Bone ALP):

The bone formation marker, Bone ALP, was measured in duplicate with the MicroVue^{TM}BAP EIA Kit (Quidel Corporation, San Diego, CA, U.S.A.). This EIA kit utilizes a monoclonal anti-Bone ALP antibody. The catalytic activity of the captured enzyme is used to measure Bone ALP activity in serum. Enzyme activity is determined spectrophotometrically and Bone ALP levels are then calculated from a calibration curve fit with a quadratic equation. Values are expressed as Units per Liter (U/L), with each unit representing one mole of p-nitrophenyl phosphate (pNPP) hydrolyzed per minute at 25°C. The range of intra-assay and inter-assay coefficient of variation are 0.6-0.7% and 2.2-7.3%, respectively. The Bone ALP assay protocol was performed as follows (Appendix I):

- Allow kit and specimens to come to room temperature.
- Add 125 µL assay Buffer to each well.
- Add 20 µL standards, controls, and samples to the appropriate well then swirl.
- Incubate for 3 hrs ± 10 min at room temperature.
- Wash 4 times with 1X wash buffer using and blot dry after the last wash.

- Add 150 µL working substrate solution.
- Incubate for 30 ± 5 minutes at room temperature.
- Add 100µL Stop Solution and Read at 405 nm with the Model 680XR Microplate Reader (Bio-Rad Laboratories, Inc., Hercules CA).

Tartrate-Resistant Acid Phosphatase 5b (TRAP5b):

The bone resorption marker, TRAP5b, was measured in duplicate using the MicroVue^{TM}TRAP5b Enzyme Immunoassay Kit (Quidel Corporation, San Diego, CA, U.S.A.). The MicroVue^{TM}TRAP5b EIA Kit detects the enzyme activity of TRAP5b based on an immune-captured enzyme assay system and the TRAP5b units are reported in U/L. The range of intra-assay coefficients of variation is 0.4-6.1%. The TRAP5b assay protocol was performed as follows (Appendix I):

- Allow specimens and kit to come to room temperature.
- Reconstitute standards and controls with 300 µL of deionized water.
- Dilute 10X Wash Buffer 1:10 with deionized water.
- Pipette 100 µL of Sample Dilutent into microplate wells.
- Pipette 50 µL of each reconstituted standard, control and sample into appropriate microplate wells.
- Seal the microwell plate with supplied plate tape cover and incubate for 60 minutes at 18-28 °C on a microplate shaker set at 550 rpm.
- After incubation, wash the microplate wells three times with a minimum of 300 µL of Wash Buffer per well using the MultiWash Microplate Washer (Tri Continent, Grass Valley, CA). After washing, tap the wells gently on a paper towel to expel any remaining liquid.
- Add 8 mL of Substrate Reconstitution Buffer to the Substrate (Prepare within 30 minutes of use).

- Pipette 100 µL of Working Substrate Solution into each well.
- Seal the microplate and mix on a microplate shaker for 30 seconds at 550 rpm. After shaking, incubate for 60 minutes in a 39°C incubator.
- Pipette 50 µL of Stop Solution into each well to stop the reaction.
- Measure absorbance at 405 nm as reference using the Model 680XR Microplate Reader (Bio-Rad Laboratories, Inc., Hercules CA).

Hormone Assays

Insulin-like Growth Factor-I (IGF-I):

IGF-I concentration was assessed by using a two-site immunoenzymometric assay (IEMA) for the quantitative determination of Insulin-like Growth Factor I (Immunodiagnostic Systems Inc, Fountain Hills, AZ).

The range of intra-assay and inter-assay coefficients of variation are 11.3-15.7% and 5.7-7.4%, respectively. The IGF-I assay protocol was performed by using the following procedure (Appendix J):

- Allow all reagents and serum to reach room temperature and mix thoroughly before use.
- Prepare labeled plastic tubes, one for each calibrator, control and sample.
- Add 25 µL of each calibrator, control or sample to appropriately labeled tubes.
- Add 100 µL of releasing reagent to each tube. Vortex all tubes. Incubate at 18-25 °C for 10 minutes.
- Add 1.0 µL of sample diluents to each tube. Vortex all tubes.
- Add 50 µL of each diluted calibrator, control, or sample to the appropriate wells of the antibody coated plate in duplicate. These should be dispensed within a period of 10 minutes to minimize drift.

- Add 200 µL of enzyme conjugate to all wells using a multichannel pipette. Incubate at 18-25 °C for between 2 hours and 2 hours 15 minutes.
- Wash all wells three times with wash solution: set plate washer to dispense at least 300 µL of wash solution per well.
- Add 200 µL of TMB substrate to all wells using a multichannel pipette. Incubate at 18-25 °C for 30 minutes.
- Add 100 µL of stop solution.
- Measure the absorbance of each well using a microplate reader within 30 minutes of adding the stop solution.

Data Analyses

All descriptive data for the dependent variables are presented as the mean ± SE. Group differences in baseline values for the dependent variables were determined by independent t-tests. If there were significant group differences at baseline, one-way analysis of covariance (ANCOVA) was used to compare group differences in the post variables using the baseline variable as a covariate. If there were no group differences at baseline, two-way mixed factorial analysis of variance (ANOVA) [Group (YE vs. CON) × Time (pre vs. post)] with repeated measures was used to analyze mean differences between groups. If a significant group × time interaction occurred, paired samples t-tests were used as post-hoc tests to determine significant time differences within each group. The percent changes in dependent variables were calculated (%Δ = [(post – pre) / pre] × 100). An independent t-test was used to examine significant group differences in the percent change variables. All statistical procedures were performed using SPSS for Windows 17.0 version (Chicago, IL). The level of significance was set at $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to examine the effects of an 8-month Yoga exercise program on bone biomarkers, areal bone mineral density (aBMD) for the total body, dual proximal femur, and lumbar spine; and volumetric bone mineral density (vBMD) for the tibia and forearm in premenopausal women. Subjects were healthy premenopausal women, between the ages of 35 and 50 years, who were randomly assigned to either the Yoga intervention group (YE) or to the control group (CON). A secondary purpose was to examine responses of IGF-I, body composition, and muscle strength (1RM) to the Yoga intervention.

Subject Characteristics

A total of 91 subjects were initially interested in participating in the study, however, 44 potential subjects were screened out due to various reasons. Sixteen out of 44 were outside of the age range, 4 were weight training prior to the study, 6 were taking birth control pills, 4 had not met the schedules, 1 did not want to participate without compensation, 7 did not respond after the first interview, 1 was doing Yoga, and 5 had had medical conditions such as high blood pressure, hypothyroidism, arthritis, or being overweight. Forty-seven subjects were enrolled at baseline, however, 13 subjects did not complete the intervention: 4 because of time commitments and 6 because of recent diagnoses of serious migraine, high blood pressure, hypothyroidism, tumor, menopausal symptoms, or chronic fatigue. One subject was excluded from the analyses due to poor attendance (below 80%). One could not be contacted, and 1 did not want to participate in post testing due to personal reasons. Thirty-four subjects (YE, n=16; CON, n=18) completed the entire 32 weeks of the study and the attendance rate of participants in YE was 92.6% for the 8 months.

Table 1 shows the baseline physical characteristics, bone-specific physical activity (BPAQ) score, calcium intake from a total frequency questionnaire, and dietary variables from 3 day dietary log for each group. There were no significant group differences for the physical characteristics, BPAQ, calcium intake, and dietary variables at baseline ($p>0.05$). One subject's 3-day dietary log in YE was missing so only 15 subjects were used for dietary variables. Subjects with a calcium intake of less than 1000 mg/day were instructed to increase their intake to at least 1000 mg/day. All subjects received the calcium recommendation sheet with their estimated daily intake (Appendix K). Two-way mixed factorial ANOVA with repeated measures detected a significant ($p<0.001$) time effect for increasing calcium intake after 8 months of intervention (YE, 1438±192: CON, 1594±181).

Table 1. Physical Characteristics for YE and CON Groups at the Beginning of Study.

	Group			
Variable	YE	n	CON	n
Age (years)	45.7±1	16	43.2±1	18
Ethnicity (A/W/O)	4/12/0	16	3/11/4	18
Height (cm)	162.8±1.3	16	160.8±1.3	18
Weight (kg)	69.71±3.32	16	70.03±2.16	18
BMI (kg/m^2)	26±1	16	27±1	18
BPAQ	22.7±6.5	16	25.7±5.7	18
Ca^{2+} Intake (mg/day)	1025±165	16	1162±156	18
Dietary Intake(a)				
Total CI (kcal)	1731.9±107.8	15	2502.4±354.1	18
Protein (g)	71.6±6	15	94.4±1	18
Carbohydrates (g)	207.4±16.1	15	312.7±46.8	18
Fat (g)	71±5.6	15	100.7±17.3	18
Vitamin D (µg)	4.4±2.1	15	6.5±1.6	18
Magnesium (mg)	211.2±31.7	15	323.7±41.4	18

Values are means ± SE. YE: Yoga Exercise, CON: Control, A: Asian, W: White, O: Other, BMI: Body Mass Index, BPAQ: Bone-Specific Physical Activity Score, Total CI: Total Caloric Intake.
a: determined by 3-day dietary logs.

Biochemical Markers of Bone Turnover Responses to Training

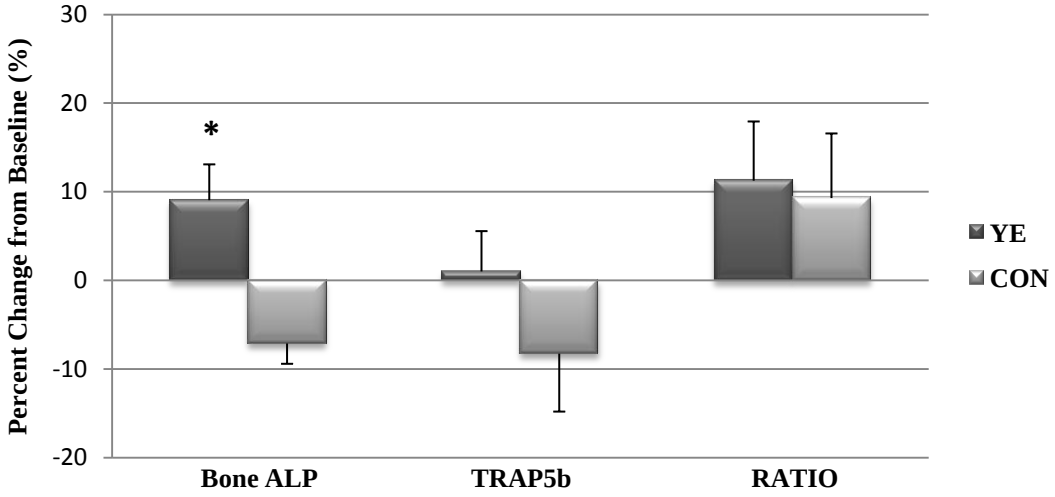
Serum Bone Alkaline Phosphatase (Bone ALP), Tartrate-Resistant Acid Phosphatase 5b (TRAP5b) and the ratio of Bone ALP to TRAP5b for each group at baseline and after post-training are shown in Table 2. There were no significant differences between groups for Bone ALP and TRAP5b at baseline. There was no significant time effect ($p>0.05$) for Bone ALP, however, a significant group x time interaction ($p<0.01$) was detected. Paired samples t-tests indicated that the CON group had a significant decrease in serum Bone ALP concentration after 8 months ($p<0.01$). No significant time or group x time interaction effects were detected for TRAP5b and the ratio of Bone ALP to TRAP5b. The Bone ALP reference range for premenopausal women 25-44 years of age is 11.6 - 29.6 (U/L). The TRAP5b reference range for premenopausal women 30 - 44 years of age is 1.5 - 4.3 (U/L). All subjects had values within the references at baseline.

Table 2. Biochemical Markers of Bone Turnover before and after Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Bone ALP (U/L)**	24.0±2.2	26.8±2.2	29.1±2.0	27.0±2.1††
TRAP5b (U/L)	2.5±0.2	2.4±0.2	2.6±0.2	2.3±0.2
Ratio	10.8±1.0	11.6±1.3	12.0±0.9	12.9±1.2

Values are means ± SE. YE: Yoga Exercise, CON: Control, Bone ALP: Bone Alkaline Phosphatase, TRAP5b: Tartrate-Resistant Acid Phosphatase 5b, Ratio: Ratio of Bone ALP to TRAP5b.
**p<0.01 Significant group x time interaction, †† p<0.01 baseline vs. post for CON.

Figure 2. Biochemical Marker Percent Change after 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control, Bone ALP: Bone Alkaline Phosphatase, TRAP5b: Tartrate-Resistant Acid Phosphatase 5b, Ratio: Ratio of Bone ALP to TRAP5b.
*p<0.05 Significant group difference.

Figure 2 shows the percent changes in serum Bone ALP, TRAP5b concentrations and the ratio of Bone ALP to TRAP5b. There were significant group differences ($p<0.05$) in percent changes for Bone ALP concentrations as the YE group had a significantly greater relative increase compared to the CON group (9.1% vs. -7.1%). There were no significant group differences ($p>0.05$) in percent changes for TRAP5b and ratio of Bone ALP to TRAP5b, but there was a trend ($p=0.061$) for TRAP5b.

Bone Mineral Density Responses to Training

Table 3 shows the baseline and post-testing BMD values at the total body, right and left hip for the total hip and femoral neck sites, trochanter, and the lumbar spine (L1-L4). At baseline, there was a significant difference ($p=0.043$) between groups for BMD at the right total hip as the CON group had a higher BMD value compared to the YE group. There were no significant group differences for total body, right (femoral neck, trochanter) left (total hip, femoral neck, trochanter), and lumbar spine (L1-L4) at baseline ($p>0.05$). Since there were significant group differences for the right total hip at baseline, one-way ANCOVA was used to detect significant differences. After using pre right total hip BMD as a covariate, one-way ANCOVA did not find significant differences ($p>0.05$). Also, no significant time and group x time interaction effects for BMD at each site were detected by two-way mixed factorial ANOVA with repeated measures ($p>0.05$).

Table 3. BMD Values before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total Body	1.169±0.021	1.163±0.019	1.184±0.015	1.184±0.017
Right				
Total Hip	0.990±0.034	0.989±0.033	1.037±0.020*	1.040±0.020
Femoral Neck	0.963±0.029	0.956±0.030	1.011±0.023	1.011±0.019
Trochanter	0.789±0.033	0.789±0.033	0.798±0.021	0.807±0.022
Left				
Total Hip	0.999±0.031	0.996±0.033	1.030±0.018	1.031±0.019
Femoral Neck	0.971±0.030	0.963±0.031	0.995±0.017	0.999±0.018
Trochanter	0.793±0.032	0.788±0.032	0.805±0.018	0.808±0.019
Spine				
L1-L4	1.210±0.037	1.210±0.038	1.233±0.027	1.231±0.026

Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g/cm^2.
*$p<0.05$ Significant group difference at baseline.

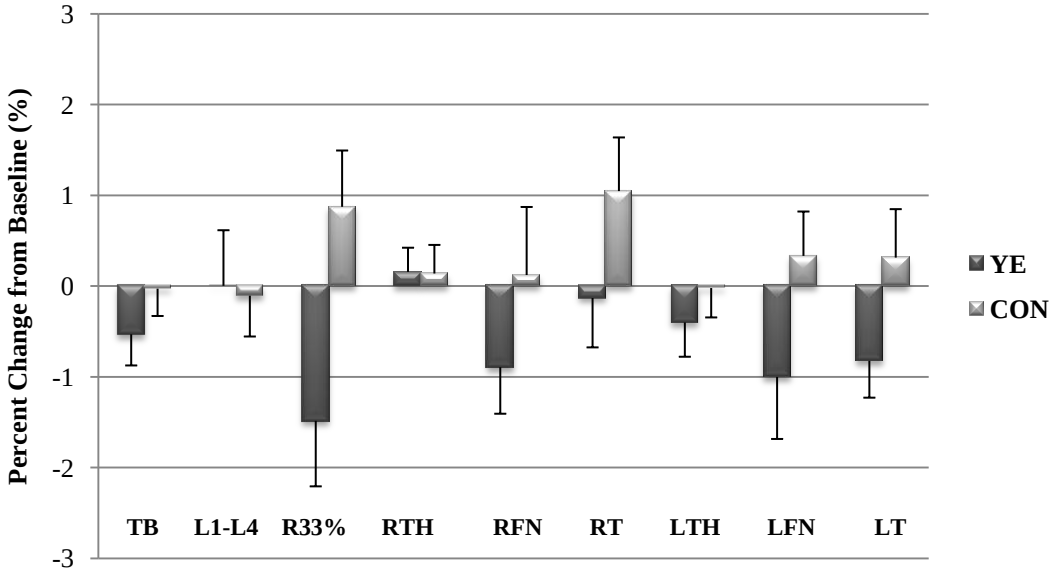
Table 4. Radius 33% BMD before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Radius 33%*	0.885±0.014	0.872±0.013	0.863±0.017	0.869±0.015

Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g/cm^2.
*p<0.05 Significant group x time interaction.

The baseline and post-testing BMD values at the radius 33% site for each group is shown in Table 4. There was no significant group difference at baseline (p>0.05). After the 8-month intervention period, there was no main time effect (p>0.05), however, a significant group x time interaction (p=0.024) was detected by two-way mixed factorial ANOVA with repeated measures. Paired samples t-tests revealed no significant time differences within each group, however, there was a negative trend (p=0.061) in YE group.

Figure 3. Total Body, Lumbar Spine, Radius 33%, Right/Left Total Hip, Femoral Neck, and Trochanter BMD Percent Change from Baseline for each Group.



Values are means ± SE. YE: Yoga Exercise, CON: Control. TB: Total body, R33%: Radius 33%, RTH: Right Total Hip, RFN: Right Femoral Neck, RT: Right Trochanter, LTH: Left Total Hip, LFN: Left Femoral Neck, LT: Left Trochanter.

There were no significant group differences in percent changes for the total body, lumbar spine (L1-L4), radius 33% , right/left total hip, femoral neck, and trochanter from baseline to post-testing (p>0.05), which are shown in Figure 3.

Bone Mineral Content Responses to Training

The baseline values of BMC for total body, hip sites (both sides total hip, femoral neck, trochanter), lumbar spine and radius 33% for each group are reported in Table 5. At baseline, independent samples t-tests detected significant differences between groups for BMC at left total hip and trochanter ($p<0.05$). For the left total hip, the CON group had significantly higher BMC than a YE group (p=0,030). For the left trochanter, however, the YE group had significantly higher BMC than the CON group (p=0.015). For total body, right total hip, femoral neck, trochanter, left femoral neck, lumbar spine, and radius 33% BMC values, there were no significant group differences at baseline ($p>0.05$).

Table 5. Baseline BMC Values for each Group.

	Group	
Variable	YE (n=16)	CON (n=18)
Total Body	2551±76	2619±80
Right		
Total Hip	29.8±1.3	30.1±0.7
Femoral Neck	4.3±0.1	4.6±0.1
Trochanter	9.0±0.6	8.4±0.3
Left		
Total Hip	30.2±1.3*	30.2±0.7
Femoral Neck	4.4±0.1	4.6±0.1
Trochanter	9.2±0.6*	8.7±0.3
Spine		
L1-L4	65.5±2.4	62.9±2.1
L2-L4	52.4±1.9	50.3±1.6
Radius 33%	2.1±0.0	2.1±0.1

Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.
*$p<0.05$ Significant group differences.

The baseline and post testing BMC values of the total body, right total hip, femoral neck and trochanter for each group are shown in Table 6. There was a significant time effect for right total hip (p=0.033) and right trochanter (p=0.016) after the 8 month intervention. The right total hip and trochanter significantly increased in both groups. For the total body and right femoral neck, no significant time or group x time interactions effects were detected by two-way mixed factorial ANOVA with

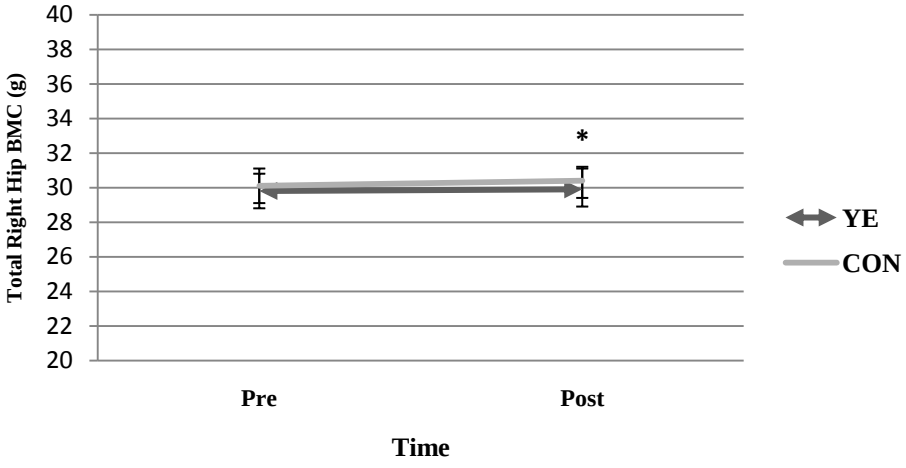
repeated measures (p>0.05). In Figures 4 and 5, baseline and post BMC values are plotted for each group at the right total hip and right trochanter, respectively.

Table 6. Total Body and Right Hip BMC before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total Body	2551±76	2573±85	2619±80	2592±70
Right				
Total Hip*	29.8±1.3	29.9±1.3	30.1±0.7	30.4±0.7
Femoral Neck	4.3±0.1	4.3±0.1	4.6±0.1	4.6±0.1
Trochanter*	9.0±0.6	9.1±0.6	8.4±0.3	8.7±0.3

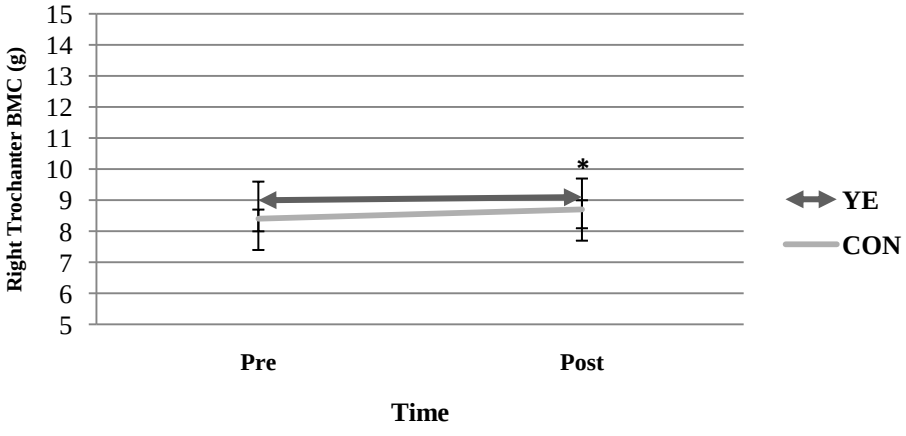
Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.
*p<0.05 Time effect.

Figure 4. Right Total Hip BMC Response to 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.
*p<0.05 Time effect.

Figure 5. Right Trochanter BMC Response to 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.
*p<0.05 Time effect.

The baseline and post testing BMC values for the left total hip, femoral neck and trochanter for each group are shown in Table 7. After correcting for baseline mean differences using one-way ANCOVA, a significant group x time interaction was detected for left total hip ($p<0.05$). Paired samples t-tests indicated that the CON group had a significant increase in left total hip after 8 months ($p=0.020$). There was no time effect or group x time interaction for left femoral neck ($p>0.05$). For left trochanter, no significant time effect was observed after running one-way ANCOVA ($p>0.05$), however, a significant group x time interaction was present ($p<0.01$). Paired samples t-tests indicated that the CON group had a significant increase in left trochanter after 8 months ($p=0.001$).

Table 7. Left Hip BMC before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Left				
Total Hip*	30.2±1.3	30.1±1.3	30.2±0.7	30.4±0.7†
Femoral Neck	4.4±0.1	4.4±0.1	4.6±0.1	4.6±0.1
Trochanter**	9.2±0.6	9.0±0.6	8.7±0.3	8.9±0.3††

Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.
*$p<0.05$ Significant group x time interaction, **$p<0.01$ Significant group x time interaction,
†$p<0.05$ Significant baseline vs. post, ††$p<0.01$ Significant baseline vs. post.

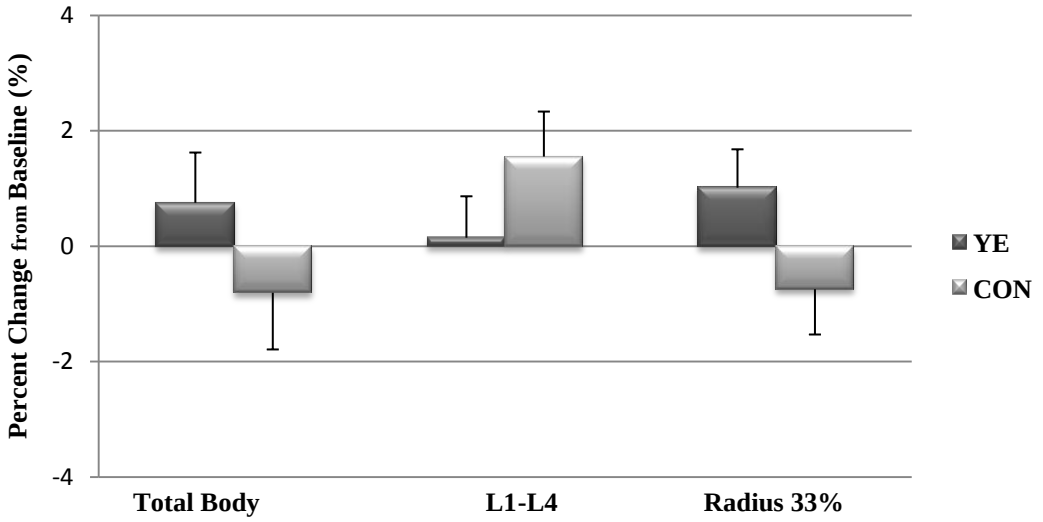
Table 8 shows the baseline and post-testing BMC values of the lumbar spine (L1-L4, L2-L4) and radius 33%. No significant time or group x time interaction effects were detected for the lumbar spine and radius 33% after 8 months of intervention ($p>0.05$).

Table 8. Lumbar Spine and Radius 33% BMC before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Spine				
L1-L4	65.5±2.4	65.6±2.5	62.9±2.1	63.7±1.9
Radius 33%	2.1±0.0	2.1±0.1	2.1±0.1	2.0±0.1

Values are means ± SE. YE: Yoga Exercise, CON: Control. All values expressed in g.

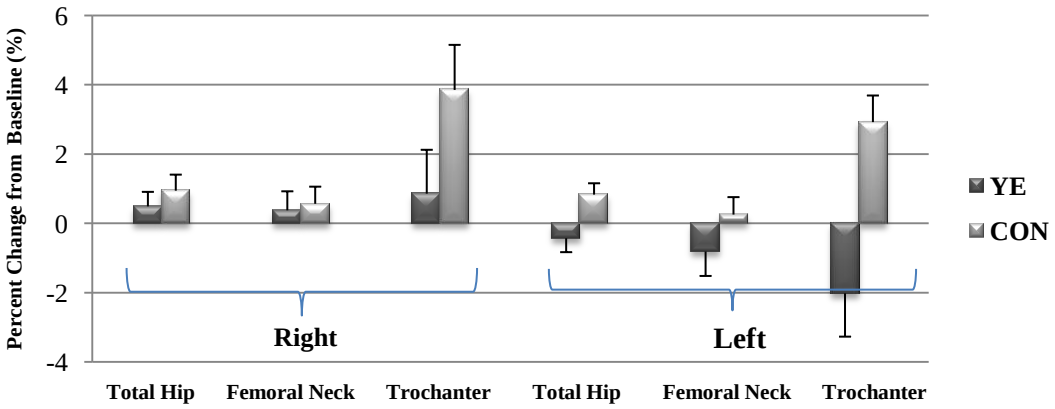
Figure 6. Total Body, Lumbar Spine, and Radius 33% BMC Percent Change from Baseline for each Group.



Values are means ± SE. YE: Yoga Exercise, CON: Control.

Percent change from baseline BMC values for the total body, lumbar spine (L1-L4) and radius 33% are shown in Figure 6. There were no significant group differences ($p>0.05$) in percent changes for the total body, lumbar spine, and radius 33%.

Figure 7. Right and Left Hip BMC Percent Change from Baseline.



Values are means ± SE. YE: Yoga Exercise, CON: Control.

Percent change from baseline BMC values for the right and left hip (total hip, femoral neck, trochanter) are reported in Figure 7. Independent samples t-test did not detect significant differences in percent changes for the right and left total hip, femoral neck, and trochanter ($p>0.05$).

Bone Characteristics' Responses to Training

In Table 9, vBMD of tibia 4% site for bone content, bone area, periosteal circumference, and Strength-Strain Index (SSI) for total and trabecular is displayed at baseline and post-training. There were no significant group differences at baseline ($p>0.05$). Two-way mixed factorial ANOVA with repeated measures did not detect any significant time or group x time interaction effects after 8 months of intervention ($p>0.05$).

Table 9. Tibia 4% Bone Characteristics before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total vBMD (mg/cm^3)	284.050±9.181	284.513±8.280	300.456±9.184	301.889±8.838
Total Bone Content (mg)	270.749±9.682	270.072±9.068	261.487±6.893	260.798±6.648
Total Bone Area (mm^2)	958.470±29.807	952.360±25.473	879.378±27.434	873.858±29.695
Trabecular vBMD (mg/cm^3)	232.481±6.940	232.800±6.959	236.678±6.523	236.311±6.305
Trabecular Content (mg)	188.164±7.077	187.316±6.475	172.594±7.149	170.331±6.443
Trabecular Area (mm^2)	813.700±28.262	807.670±23.519	733.262±27.941	726.231±29.372
Periosteal Circumference (mm)	109.549±1.703	109.249±1.468	104.905±1.638	104.540±1.759

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density.

Table 10 shows the baseline and post-testing vBMD values for tibia 38%. There were no significant group differences at baseline ($p>0.05$). No significant time and group x time interaction effects were detected by two-way repeated measures ANOVA ($p>0.05$).

Table 10. Tibia 38% Bone Characteristics before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total vBMD (mg/cm^3)	938.181±12.866	936.043±12.698	919.261±14.661	919.833±14.480
Total Bone Content(mg)	330.573±9.127	330.227±9.081	322.475±7.001	323.098±6.811
Total Bone Area (mm^2)	353.150±10.374	353.530±10.248	351.227±6.833	351.707±6.722
Cortical vBMD (mg/cm^3)	1189.613±5.972	1187.066±5.713	1182.600±6.917	1182.850±7.425
Cortical Content (mg)	315.101±8.959	314.688±8.919	310.614±8.054	309.617±7.320
Cortical Area (mm^2)	265.130±8.028	265.310±7.893	261.093±5.813	261.591±5.627
Cortical Thickness (mm)	5.325±0.126	5.325±0.125	5.249±0.133	5.254±0.128
Periosteal Circumference (mm)	66.510±0.976	66.548±0.963	66.380±0.655	66.411±0.645
SSI	1450.300±57.070	1447.326±56.831	1442.429±41.487	1436.778±38.426

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, SSI: Strength-Strain Index.

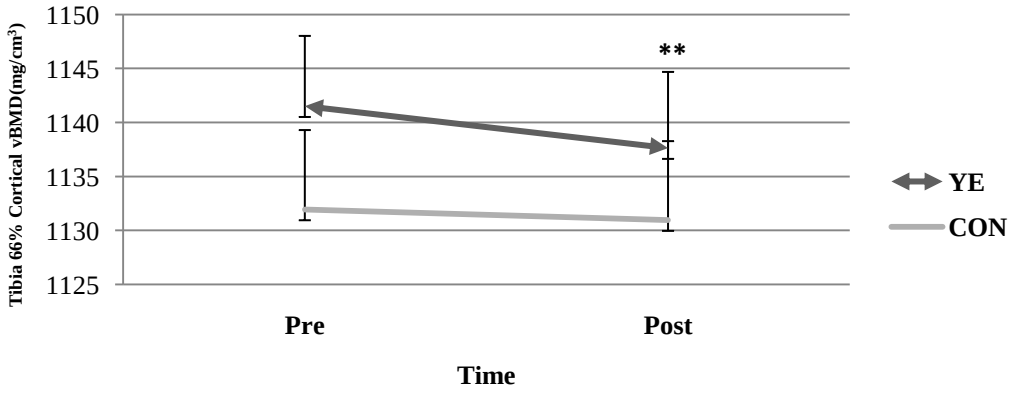
Table 11 shows the baseline and post testing vBMD values of the tibia 66% for each group. At baseline, no significant differences in vBMD at each bone site existed between groups. Two-way mixed factorial ANOVA with repeated measures revealed a significant ($p<0.01$) time effect for cortical vBMD at the tibia 66% site; cortical vBMD in both groups significantly decreased after 8 months of intervention (Figure 8).

Table 11. Tibia 66% Bone Characteristics before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total vBMD (mg/cm^3)	656.188±16.543	655.313±15.945	659.072±13.664	660.344±14.068
Total Bone Content(mg)	346.923±9.760	346.827±9.710	343.097±7.256	343.113±7.345
Total Bone Area (mm^2)	532.990±18.993	533.110±18.373	522.462±11.244	521.253±10.389
Cortical vBMD** (mg/cm^3)	1141.506±6.509	1137.625±7.054	1131.944±7.352	1130.956±7.314
Cortical Content (mg)	308.619±8.476	309.198±8.448	306.374±7.003	306.362±7.306
Cortical Area (mm^2)	270.620±7.918	272.070±7.932	270.356±5.211	270.578±5.553
Cortical Thickness (mm)	3.916±0.114	3.938±0.111	3.954±0.083	3.964±0.092
Periosteal Circumference (mm)	81.648±1.445	81.669±1.399	80.948±0.872	80.865±0.806
SSI	2226.533±95.705	2219.617±92.480	2158.143±61.381	2154.106±57.356

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density. SSI: Strength-Strain Index. **p<0.01 Time effect.

Figure 8. Tibia 66% Cortical vBMD Response to 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control. **p<0.01, Time effect.

Table 12. Percent Changes in Tibia 4%, 38%, and 66% Bone Characteristics.

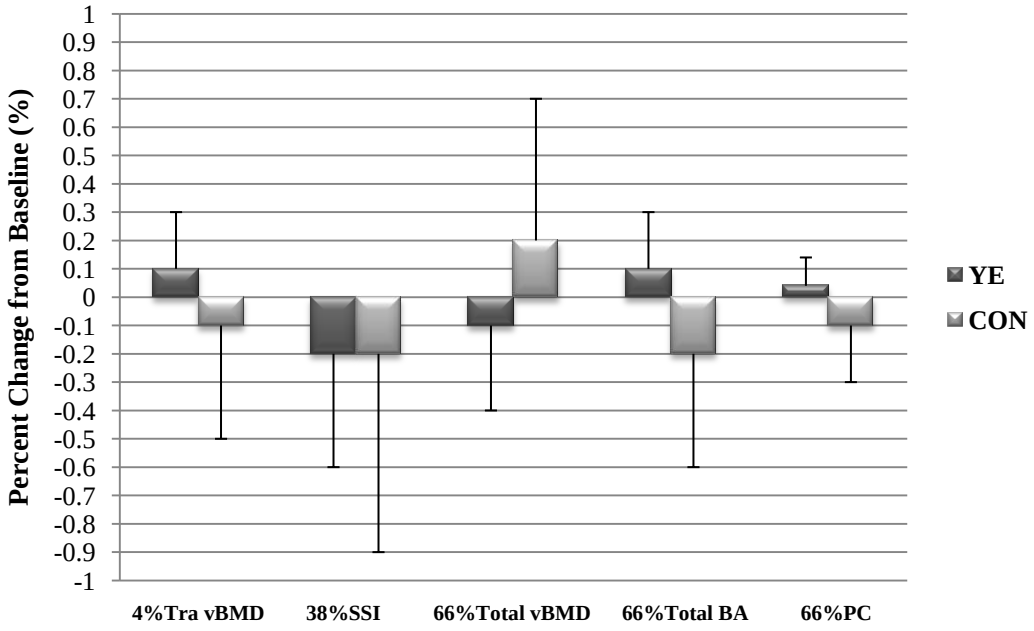
	Group					
	YE (n=16)			CON (n=18)		
Variable	4%	38%	66%	4%	38%	66%
Total vBMD (mg/cm^3)	0.3±0.7	-0.2±0.1	-0.1±0.3*****	0.6±1.1	0.1±0.1	0.2±0.5*
Total Bone Content(mg)	-0.1±0.9	-0.1±0.2	-0.0±0.1	-0.1±0.9	0.2±0.2	-0.0±0.3
Total Bone Area (mm^2)	-0.3±1.3	0.1±0.2	0.1±0.2*****	-0.5±1.5	0.2±0.2	-0.2±0.4*****
Cortical vBMD (mg/cm^3)	--	-0.2±0.1	-0.3±0.1	--	0.0±0.1	-0.1±0.1
Cortical Content (mg)	--	-0.1±0.2	0.2±0.2	--	-0.2±0.6	-0.0±0.3
Cortical Area (mm^2)	--	0.1±0.3	0.5±0.2	--	0.2±0.3	0.0±0.3
Cortical Thickness(mm)	--	0.0±0.3	0.6±0.3	--	0.1±0.3	0.2±0.5
Trabecular vBMD(mg/cm^3)	0.1±0.2*****	--	--	-0.1±0.4*****	--	--
Trabecular Content(mg)	-0.1±1.8	--	--	-0.7±2.1	--	--
Trabecular Area (mm^2)	-0.3±1.6	--	--	-0.7±1.9	--	--
Periosteal Circumference (mm)	-0.2±0.6	0.1±0.1	0.0±0.1*****	-0.3±0.8	0.0±0.1	-0.1±0.2*****
SSI	--	-0.2±0.4*****	-0.2±0.3	--	-0.2±0.7*****	-0.1±0.4

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, SSI: Strength-Strain Index.*p<0.05 Significant group differences.

Percent change from baseline vBMD values for the tibia 4%, 38%, and 66% are reported in Table 12. Independent samples t-tests detected significant differences in percent changes for the total vBMD at the tibia 66% (p=0.037). The YE group showed a decrease in total vBMD while the CON group showed an increase in total vBMD at the tibia 66%. There were significant group differences (p=0.018) in percent changes for the total bone area at the tibia 66%. The result showed that the YE group significantly increased when compared to the CON group (0.1±0.2 vs. -0.2±0.4). Also, independent samples t-test detected significant differences in percent changes for the trabecular vBMD at the tibia 4% (p=0.034). The YE group showed an increase in trabecular vBMD while the CON group showed a decrease in trabecular vBMD at the tibia 4%. There were significant group differences (p=0.018) in percent changes for preriosteal circumference, showing that the CON group significantly decreased

compared to the YE group. Finally, independent samples t-tests detected significant differences in percent changes for SSI at the tibia 38% (p=0.021). SSI of the tibia 38% in both groups significantly decreased after 8 months of intervention. Percent change from baseline vBMD values for tibia 66% total vBMD, total bone area, periosteal ciucumference, and tibia 4% trabecular vBMD, and tibia 38% SSI are shown in Figure 9.

Figure 9. Tibia 4% Trabecular vBMD, Tibia 38% SSI and Tibia 66% Total vBMD, Total Bone area, Periosteal Circumference Percent Change from Baseline.



Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, Tra vBMD: Trabecular vBMD, SSI: Strength Strain Index, Total BA: Total Bone Area, PC: Periosteal Circumference. *p<0.05 Significant group differences.

Table 13 shows the means ± SE for the baseline vBMD values at the radius 4% and 66% for each group. One subject in the YE group did not participate in the radius 4% scan due to a previous fracture. Independent samples t-tests did not reveal any significant differences ($p>0.05$) between groups for vBMD at each site except for the total vBMD at the radius 4%. However, the YE group had a significantly higher BMD value compared to the CON group at baseline ($p<0.001$).

Table 13. Baseline Radius 4% and 66% Bone Characteristics.

	Group			
	YE		CON	
Variable	4% (n=15)	66% (n=16)	4% (n=18)	66% (n=18)
Total vBMD (mg/cm^3)	360.567±15.370†	786.538±28.275	288.541±33.678†	723.594±23.241
Total Bone Content(mg)	96.023±3.536	96.506±4.872	95.473±2.754	92.539±3.411
Total Bone Area (mm^2)	270.091±10.754	124.300±6.583	264.560±13.772	129.049±4.704
Cortical vBMD (mg/cm^3)	--	1148.975±7.954	--	1124.217±9.267
Cortical Content (mg)	--	87.426±4.427	--	83.277±3.424
Cortical Area (mm^2)	--	76.010±3.689	--	74.044±2.976
Cortical Thickness(mm)	--	2.415±0.108	--	2.250±0.089
Trabecular vBMD (mg/cm^3)	214.740±9.779	--	218.439±9.667	--
Trabecular Content(mg)	42.523±3.072	--	41.450±3.026	--
Trabecular Area (mm^2)	197.920±11.589	--	193.298±15.224	--
Periosteal Circumference (mm)	58.102±1.143	39.313±1.049	57.336±1.478	40.152±0.748
SSI	--	298.041±19.956	--	299.588±14.764

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, SSI: Strength Strain Index. †$p<0.001$ Significant group differences.

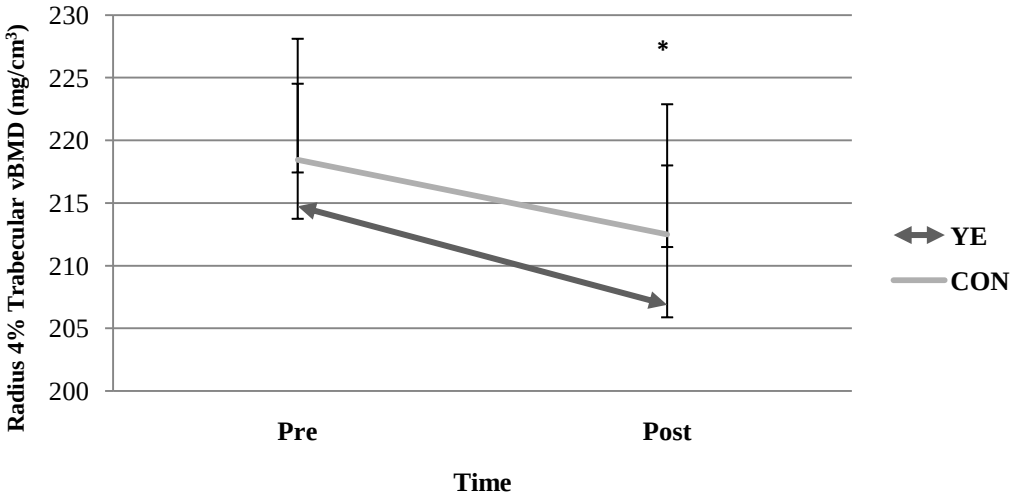
Table 14 presents the baseline and post testing vBMD values of the radius 4% for each group. Since there were significant group differences for the radius 4% total vBMD at baseline, one-way ANCOVA was used to detect significant differences. However, one- way ANCOVA did not find group differences ($p>0.05$). There was significant time effect for the trabecular vBMD at the radius 4% ($p=0.044$). After 8 months of intervention, the trabecular vBMD significantly decreased in both groups. In Figure 10, the trabecular vBMD is plotted by time for each group. There were no significant time or group x time interaction effects for the rest of variables at the radius 4% ($p>0.05$).

Table 14. Radius 4% Bone Characteristics before and after 8 Months of Training.

	Group			
	YE (n=15)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total vBMD (mg/cm^3)	360.567±15.370	379.900±22.197	288.541±33.678	299.348±33.983
Total Bone Content (mg)	96.023±3.536	93.471±3.745	95.473±2.754	94.525±2.982
Total Bone Area (mm^2)	270.091±10.754	254.069±13.299	264.560±13.772	252.622±10.014
Trabecular vBMD* (mg/cm^3)	214.740±9.779	206.880±11.121	218.439±9.667	212.489±10.392
Trabecular Content (mg)	42.523±3.072	38.209±3.847	41.450±3.026	37.822±2.460
Trabecular Area (mm^2)	197.920±11.589	182.069±14.337	193.298±15.224	179.716±10.610
Periosteal Circumference (mm)	58.102±1.143	56.220±1.512	57.336±1.478	56.156±1.111

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density.
*$p<0.05$ Time effect.

Figure 10. Radius 4% Trabecular vBMD Response to 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, *p<0.05, Time effect.

In Table 15, the radius 66% vBMD data at baseline and post-testing are shown. There were no significant time or group x time interaction effects for total vBMD, bone content, bone area, cortical thickness, periosteal circumference, and SSI for total and cortical ($p>0.05$).

Table 15. Radius 66% Bone Characteristics before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total vBMD (mg/cm^3)	786.538±28.275	781.244±30.927	723.594±23.241	721.978±30.512
Total Bone Content (mg)	96.506±4.872	96.656±4.671	92.539±3.411	91.034±3.622
Total Bone Area (mm^2)	124.300±6.583	125.670±6.325	129.049±4.704	127.956±5.029
Cortical vBMD (mg/cm^3)	1148.975±7.954	1142.638±10.223	1124.217±9.267	1125.600±9.625
Cortical Content (mg)	87.426±4.427	87.772±4.417	83.277±3.424	82.206±3.961
Cortical Area (mm^2)	76.010±3.689	76.710±3.637	74.044±2.976	72.809±3.258
Cortical Thickness (mm)	2.415±0.108	2.431±0.117	2.250±0.089	2.243±0.120
Periosteal Circumference (mm)	39.313±1.049	39.545±1.013	40.152±0.748	39.967±0.789
SSI	298.041±19.956	299.884±18.454	299.588±14.764	294.376±14.214

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density.

Percent change from baseline vBMD values for the radius 4%, and 66% are reported in Table 16. Independent samples t-tests did not detect any significant differences in percent changes for the vBMD variables at the radius 4% and 66% ($p>0.05$).

Table 16. Percent Changes in Radius 4% and 66% Bone Characteristics.

	Group			
	YE		CON	
Variable	4% (n=15)	66% (n=16)	4% (n=18)	66% (n=18)
Total vBMD (mg/cm^3)	5.6±4.2	-0.6±2.1	5.2±4.4	-0.6±1.7
Total Bone Content(mg)	-2.6±1.4	0.4±0.7	-0.9±1.6	-1.7±1.5
Total Bone Area (mm^2)	-5.5±4.3	1.5±2.0	-2.1±4.0	-0.7±1.8
Cortical vBMD (mg/cm^3)	--	-0.6±0.6	--	0.1±0.6
Cortical Content (mg)	--	0.6±1.5	--	-1.6±2.1
Cortical Area (mm^2)	--	1.1±1.2	--	-1.8±2.0
Cortical Thickness(mm)	--	0.9±2.5	--	-0.8±2.6
Trabecular vBMD (mg/cm^3)	-4.0±2.4	--	-2.6±2.4	--
Trabecular Content(mg)	-8.9±8.7	--	-1.5±8.5	--
Trabecular Area (mm^2)	-7.0±7.0	--	-0.9±6.5	--
Periosteal Circumference (mm)	-3.1±2.2	0.7±1.0	-1.4±2.1	-0.4±0.9
SSI	--	1.2±1.5	--	-1.4±1.8

Values are means ± SE. YE: Yoga Exercise, CON: Control. vBMD: volumetric Bone Mineral Density, SSI: Strength Strain Index.

IGF-I Response to Training

There was no significant difference between groups for Insulin-like Growth Factor-I (IGF-I) at baseline ($p>0.05$). Mean serum IGF-I concentrations for each group at baseline and post training are shown in Table 17. There was significant time effect ($p<0.01$) for IGF-I, as it decreased after training for both groups. However, no significant group x time interaction was detected by two-way mixed factorial ANOVA with repeated measures ($p>0.05$).

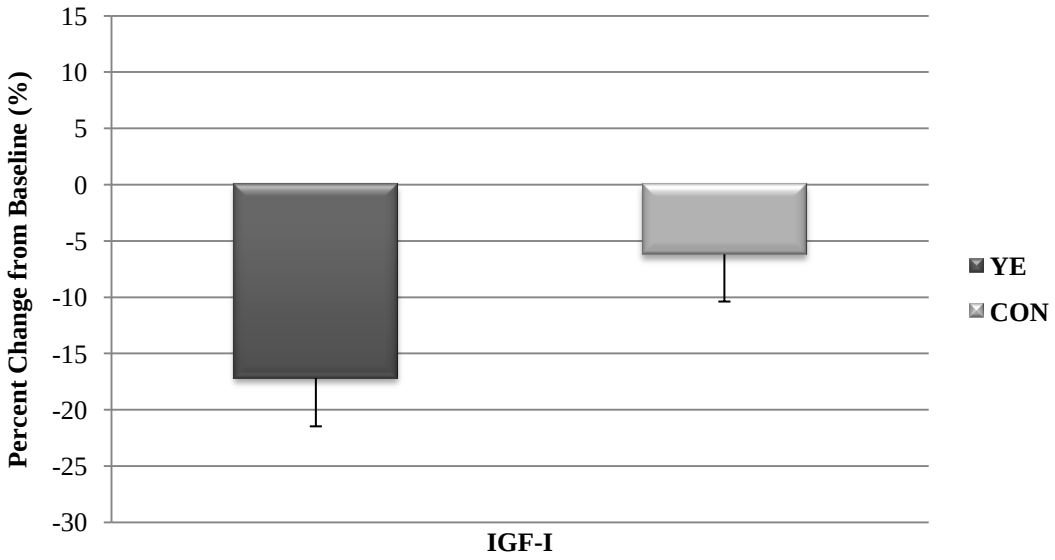
Table 17. IGF-I before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
IGF-I (ng/mL)**	94.1±7.4	76.7±7.5	95.2±7.0	88.9±7.1

Values are means ± SE. YE: Yoga Exercise, CON: Control. IGF-I: Insulin-like Growth Factor-I.
**p<0.01Time effect.

Figure 11 shows the percent changes in serum IGF-I concentrations from the baseline to post-testing. Independent samples t-tests did not detect significant differences in percent changes for IGF-I between groups ($p>0.05$).

Figure 11. IGF-I Percent Change from Baseline.



Values are means ± SE. YE: Yoga Exercise, CON: Control. IGF-I: Insulin-like Growth Factor-I.

Body Composition Changes at Baseline and After Training

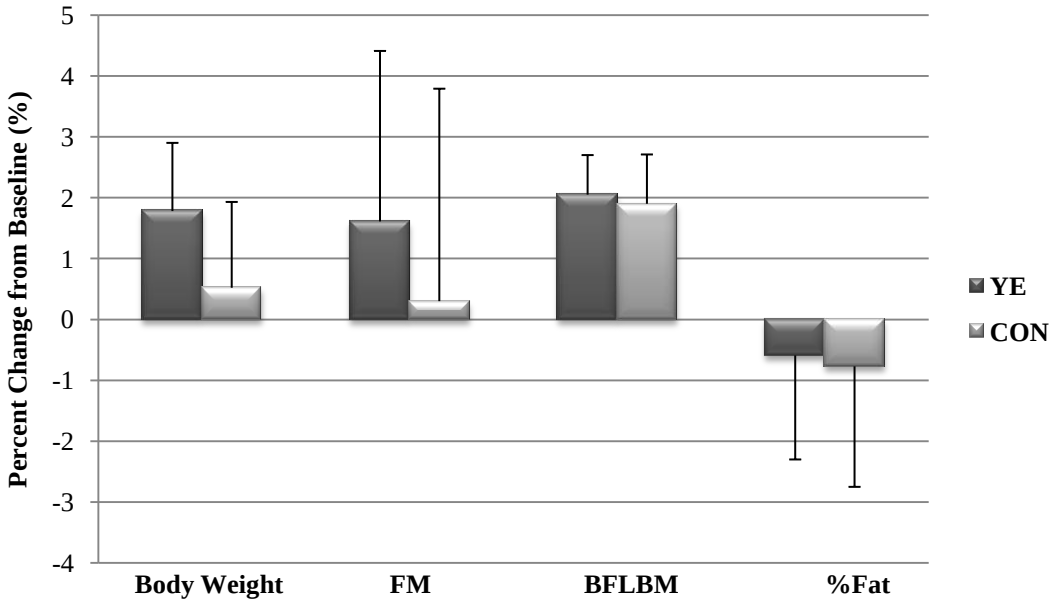
Table 18 shows the baseline and post testing body composition changes for each group. At baseline, there were no significant differences for weight, total fat mass, and bone free lean body mass (BFLBM) between the two groups. However, there was a significant group difference for %fat at baseline ($p=0.046$). It showed that %fat in CON group was greater than in YE group. One-way ANCOVA revealed that there were no group differences for %fat ($p>0.05$). A significant time effect for BFLBM was detected ($p<0.01$). The result showed BFLBM in both groups significantly increased after 8 months of intervention. There were no significant time or group x time interaction effects for weight and total FM ($p>0.05$).

Table 18. Body Composition Changes at baseline and after Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Weight (kg)	69.7±3.3	71.0±3.5	70.0±2.2	70.1±1.9
Total FM(kg)	27.8±2.2	28.5±2.6	28.2±2.0	27.7±1.8
BFLBM(kg)**	38.8±1.3	39.5±1.2	38.6±0.8	39.3±0.8
% Fat*	39.4±1.6	39.3±1.9	39.8±1.9	39.2±1.8

Values are means ± SE. YE: Yoga Exercise, CON: Control. Total FM: Total Fat Mass, BFLBM: Bone Free Lean Body Mass. * $p<0.05$ baseline, ** $p<0.01$ Time effect.

Figure 12. Percent Changes in body weight, total FM, BFLBM, and %fat.



Values are means ± SE. YE: Yoga Exercise, CON: Control. Total FM: Total Fat Mass, BFLBM: Bone Free Lean Body Mass.

Independent samples t-tests did not detect significant differences in percent changes for body weight, total FM, BFLBM, and %fat after 8 months of intervention ($p>0.05$) (Figure 12).

BPAQ Scores Measured for 8 months

Total and current BPAQ scores are shown in Table 19. At baseline, there was no significant group difference for total BPAQ scores between groups (p=0.970). After the 8 month intervention, there was a significant time effect as current BPAQ scores significantly decreased in both groups (p=0.024). No group x time interaction was detected (p>0.05).

Table 19. Total and Current BPAQ Scores in Pre and Post Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Total BPAQ	22.73±6.54	--	25.65±5.70	--
Current BPAQ	3.59±2.32	1.32±0.43	5.68±2.2	0.88±0.40

Values are means ± SE. YE: Yoga Exercise, CON: Control. BPAQ: Bone-Specific Physical Activity Score *p<0.05 Time effect.

Table 20 shows BPAQ scores at 2 month intervals in the CON group for 8 months. One-way repeated measures ANOVA did not detect BPAQ scores for time differences (2 vs. 4 vs. 6 vs. 8 month) (p>0.05).

Table 20. BPAQ Scores Measured at 2-Month Intervals for CON Group

	Group			
	CON (n=18)			
Variable	2 month	4 month	6 month	8 month
BPAQ	1.51±0.59	2.70±1.08	1.02±0.35	0.88±0.40

Values are means ± SE. YE: Yoga Exercise, CON: Control. BPAQ: Bone-Specific Physical Activity Score.

Muscle Strength Response to Training

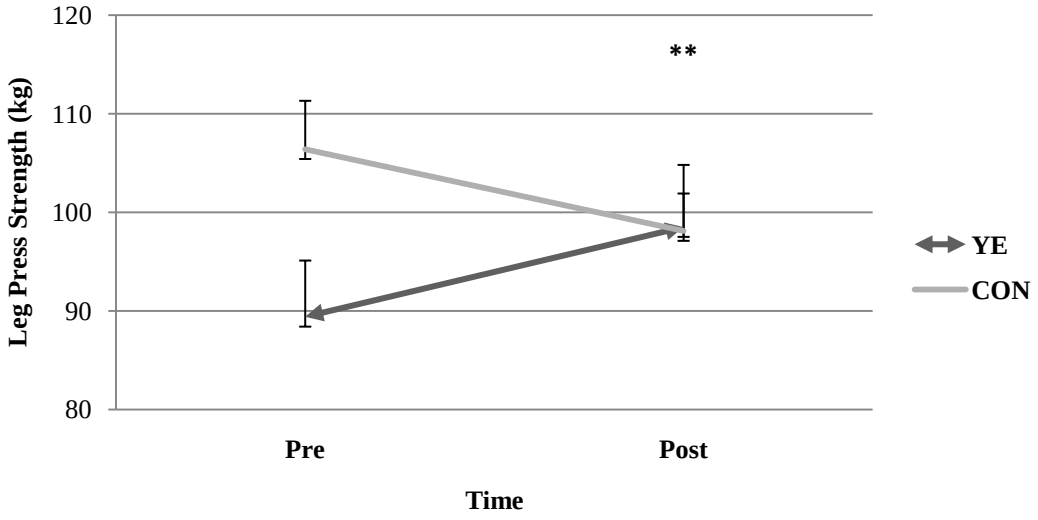
Table 21 shows the baseline and post testing muscle strength 1 repetition maximum (1RM) values for upper body (lat pull down, shoulder press, biceps curl) and for lower body (leg press, knee extension, knee flexion). At baseline, there were significant group differences in shoulder press ($p<0.05$) as the CON group was stronger than the YE group for this exercise. After adjusting baseline mean differences for shoulder press, one-way ANCOVA did not detect group differences ($p<0.01$). Two-way mixed factorial ANOVA with repeated measures detected a significant time effect ($p=0.011$) for lat pull down strength after 8 months of intervention, which significantly decreased lat pull down muscle strength in both groups. For leg press, significant group x time interaction effects were detected ($p=0.001$). Paired samples t-tests indicated that leg press strength significantly ($p<0.05$) increased in YE group, whereas the CON group slightly decreased. In Figure 13, leg press strength is plotted by time for each group. There were no time or group x time interaction effects were observed for biceps curl, knee extension, and knee flexion ($p>0.05$).

Table 21. Muscle Strength before and after 8 Months of Training.

	Group			
	YE (n=16)		CON (n=18)	
Variable	Baseline	Post	Baseline	Post
Upper Body				
Lat Pull Down**[1]	34.7±2.1	33.7±2.1	40.7±1.6	37.7±1.5
Shoulder Press	31.2±2.6	30.3±2.7	37.8±1.3*[2]	36.9±1.5
Biceps Curl	16.3±1.2	16.5±1.4	17.6±1.1	16.6±1.0
Lower Body				
Leg Press**[3]	89.4±5.7	98.5±6.3	106.4±4.9	98.1±3.8
Knee Extension	42.9±3.4	43.8±3.4	44.9±2.4	45.7±1.8
Knee Flexion	44.1±2.4	47.0±2.1	48.0±1.7	47.5±1.6

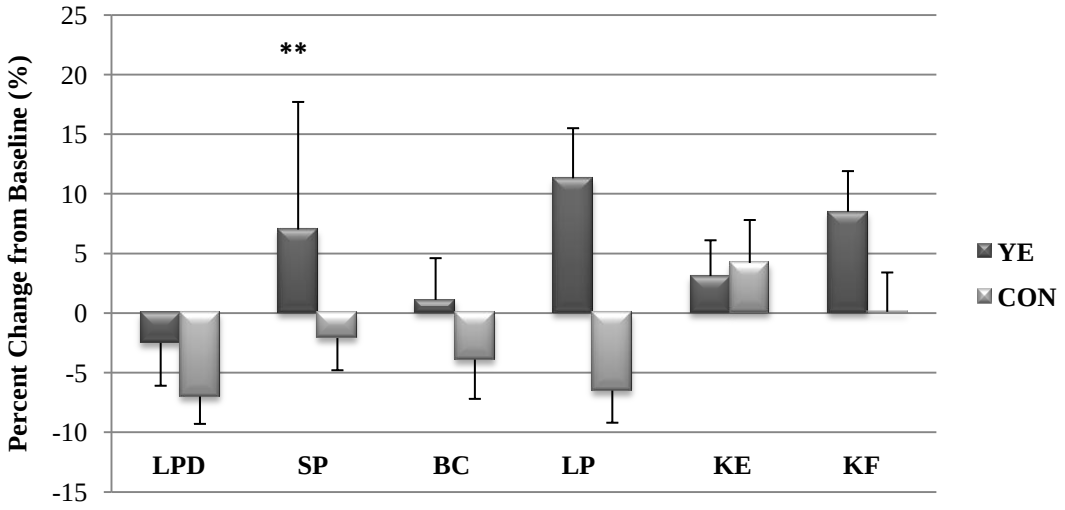
Values are means ± SE. YE: Yoga Exercise, CON: Control.
[1]*p<0.05 Time effect. [2]*p<0.05 Baseline group difference.[3]**p<0.01 Significant group x time effect,

Figure 13. Leg Press Strength Response to 8 Months of Training.



Values are means ± SE. YE: Yoga Exercise, CON: Control.
**p<0.01, Significant group x time interaction.

Figure 14. Percent Changes in Muscle Strength for each Group.



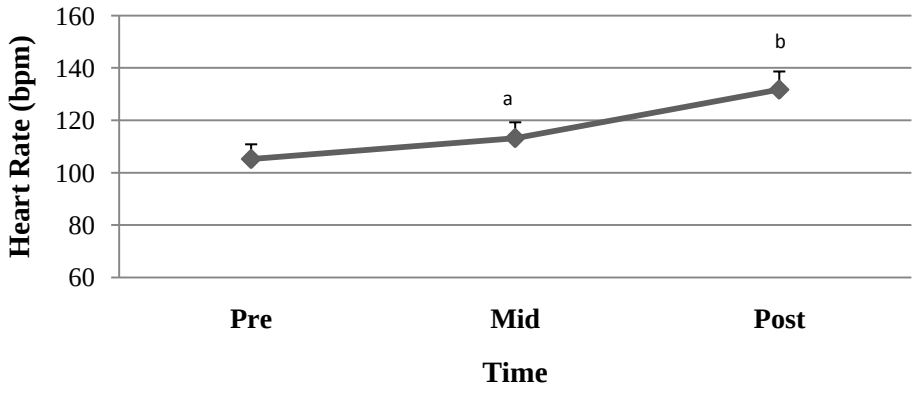
Values are means ± SE. YE: Yoga Exercise, CON: Control, LPD: Lat Pull Down, SP: Shoulder Press, BC: Bicep curl, LP: Leg Press, KE: Knee Extension, KF: Knee Flexion,
**p<0.01 Significant group difference.

Figure 14 shows the percent changes in lat pull down, shoulder press, biceps curl, leg press, knee extension, and knee flexion. There were significant group differences ($p<0.01$) in percent changes for shoulder press as the YE group had a significantly greater relative increase compared to the CON group ($7.0\pm10.7\%$ vs. $-2.1\pm2.7\%$). There were no significant percent changes in LPD. BC, LP, KE, and KF from baseline ($p>0.05$).

Heart Rate and the Rating of Perceived Exertion

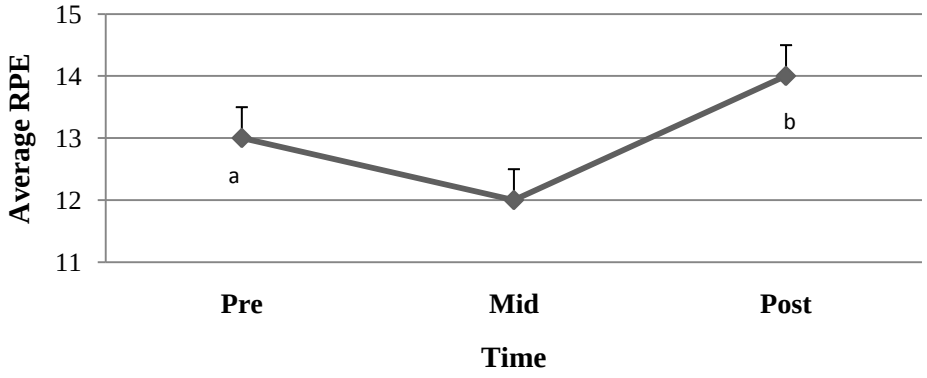
Subjects in the YE group recorded their heart rate (HR) using the palpation method for 15 seconds and the Rating of Perceived Exertion (RPE) after sun salutations every Wednesdays. One-way repeated measures ANOVA detected a significant time effect ($p<0.001$) as subjects' HR after sun salutations significantly increased for each time point. Pairwise comparison tests detected significant HR for time differences (pre vs. mid and mid vs. post)($p<0.001$). Figure 15 shows the means of the pre-, mid-, and post HR after sun salutations. In Figure 16, the RPE is plotted by the means of RPE score for each time point. Significant time effects were detected (pre vs. post, $p=0.045$ and mid vs. post, $p=0.005$)(RPE scores, Appendix E).

Figure 15. Heart Rate Changes in pre-, mid-, and post training after sun salutations.



Values are means ± SE. a $p<0.001$(pre vs. mid), b $p<0.001$ (mid vs. post).

Figure 16. RPE Changes in pre-, mid-, and post training after sun salutations.



Values are means ± SE. a $p<0.05$(pre vs. post), b $p<0.01$ (mid vs. post).

DISCUSSION

The current study examined the effects of an 8 month Yoga exercise program on bone biomarkers, areal bone mineral density (aBMD) for the total body, dual proximal femur, and lumbar spine and volumetric bone mineral density (vBMD) for the tibia and forearm in premenopausal women. According to the ACSM Position Stand for bone health (67), high intensity weight bearing endurance or resistance exercise has beneficial effects on bone health in middle-aged and older people. Although Yoga exercise has potential as an alternative way to increase BMD, the underlying mechanisms and scientific evidence are currently lacking for this type of intervention. To our knowledge, this is the first report of randomized controlled trial to determine how Yoga exercise affects bone metabolism and hormone responses in premenopausal women.

Among non-traditional forms of exercise for bone health, Tai Chi (a traditional Chinese martial art) has been recommended for osteoporotic women as a safe and effective exercise for bone density maintenance. Previous studies have indicated that regular Tai Chi exercise had beneficial effects on BMD (98) and Bone ALP (116). Both Tai Chi and Yoga have similar characteristics in terms of coordinated movements with deep breathing and they are moderate intensity in nature. In contrast with previous Tai Chi studies (98, 116), the results of a cross-sectional study (127) suggested that Yoga did not provide a sufficient stimulus to increase BMD at any site. In my study, an 8 month Yoga intervention program was designed to improve BMD following traditional Ashtanga Yoga style, referred as “power Yoga.” This study is unique because of the intensity of the intervention which was determined by the number of sun salutations and was gradually increased each month. The findings of this study indicated that Yoga intervention did not either increase

aBMD and vBMD or attenuate the rate of bone loss for each site. However, Yoga was effective for increasing the bone formation marker and leg press strength. Therefore, regular long term Ashtanga Yoga exercise could be an alternative exercise to increase bone formation and leg press muscle strength in premenopausal women.

Biochemical Markers of Bone Turnover

Biochemical markers of bone remodeling have been used to measure bone metabolism (17, 74, 129) and provide a dynamic measurement of skeletal status (81). When bone deposition occurs at a higher rate than bone resorption, bone growth occurs, resulting in thickening of bone. Conversely, when the rate of resorption is higher than that of deposition, metabolic bone diseases such as osteoporosis may result. High levels of bone turnover markers are associated with an increased risk of fracture (105), while reduced bone turnover is associated with therapeutic efficacy of bone resorption inhibitors (25). Therefore, the ratio of bone formation to bone resorption may better reflect bone turnover imbalance than one marker alone.

My study measured bone alkaline phosphatase (Bone ALP), a marker of bone formation, and Tartrate-Resistant Acid Phosphatase 5b (TRAP5b), a marker of bone resorption, at baseline and after an 8 month Yoga training intervention. The findings of this study showed that Bone ALP was significantly increased in YE group while CON group decreased (9.1% vs. -7.1%). The marker of bone resorption, TRAP5b did not change after the 8 months. One of our key research questions was if the intensity of Yoga exercise could generate mechanical loading of the skeleton to increase bone mass in premenopausal women. It has been well known that high-impact exercise or power training such as jumping or weightlifting leads to osteogenic responses in premenopausal women (67, 144). It is interesting to note that an 8 month Ashtanga Yoga exercise was able to generate mechanical loading forces to elicit increases in

bone formation. The increase in the bone formation marker could be due to dynamic movements in a variety of unusual directions such as simple back bending or twist. Also, it has been reported that oxidative stress increases differentiation and function of osteoclasts (36), whereas it compromises osteoblast differentiation (5), resulting in bone loss. Mody et al. (88) suggested that oxidative stress is able to inhibit bone cell differentiation of a preosteoblastic cell line (MC3T3-E1) and of marrow stromal cell line (M2-10B4). Sinha et al. (121) found that a 6 month intervention Yoga training in healthy male volunteers could maintain or improve antioxidant levels compared to the running plus flexibility training group. These results showed that reduced glutathione level increased significantly ($p<0.05$) in the Yoga group, whereas glutathione reductase activity increased in the control group. We did not measure oxidative stress levels, but it is possible that reduced oxidative stress might affect increases in bone formation in the current study.

No previous studies involving bone metabolism and Yoga exercise have been reported to date for premenopausal women. However, there are studies regarding the effects of alternative exercises in promotion of bone health on bone metabolic markers in postmenopausal women. A recent study by Phoosuwan et al. (95) investigated the effects of the weight-bearing Yoga training on bone markers in postmenopausal women. They found that 3 times a week weight-bearing Yoga training for 12 weeks had a positive effect on slowing down the bone resorption rate but had no effect on bone formation. Compared to my study, they trained with only 5 weight-bearing Yoga postures (tree pose, downward facing dog pose, warrior III, triangle pose and half mood pose) 3 times of 6 sets for each session. We trained the subjects twice a week one day apart for 8 months and various Ashtanga postures were performed. Also, Yoga session intensity was gradually increased by the number of

sun salutations each month. These two Yoga training programs resulted in different effects on bone turnover markers.

Similar to our results, a 6 month Tai Chi training protocol significantly increased Bone ALP after 6 weeks and the Tai Chi group exhibited a greater increase in serum Bone ALP than the resistance training group (116). There was an increase of serum pyridinoline (PYD), a marker of bone resorption, in the resistance group only, not in the Tai Chi group. In addition, they found the BAP/PYD ratio was higher than baseline only in the Tai Chi group. They concluded that these findings support Tai Chi as being beneficial for increasing bone formation in the elderly. Both Tai Chi and Yoga emphasize breathing and stretching, but Tai Chi is more likely to involve moving forms, while Yoga has many seated or lying postures. In Yoga, a series of sun salutations is a dynamic moving form and includes jumping and coordinated movements. These similar moving forms may lead to increases in bone formation markers.

In the current study, there was no change in bone resorption marker after 8 months. This result is similar to the findings of previous intervention studies (33, 117).

Consistent with previous findings, Adami et al. (2) reported that the bone formation markers (N-terminal propeptide of type I procollagen, PINP: Osteoclacin, OC), but not the bone resorption marker (carboxy-terminal collagen crosslinks, CTX), were found to be significantly associated with the level of physical activity. The same researchers conducted an intervention study (2). Twenty-four healthy sedentary premenopausal women participated in exercise training sessions 3 or 4 days each week for 4 weeks and training sessions consisted of running, walking on a treadmill, step-ups and stair climbing. The intensity of exercise was gradually increased over the 4 weeks of the program. These results showed that serum OC and PINP rose

significantly, by 25%, in the active group but no changes were observed in CTX levels in both groups. Based on previous studies, I conclude that changes in physical activity are associated with a clear effect on bone formation markers, not resorption markers.

Bone Mineral Density

The present study did not find any significant BMD differences in the total body, lumbar spine, and dual femur sites between groups. At baseline, there was a significant difference between groups for BMD at the right total hip as the CON group had a higher BMD value compared to the YE group (0.990 ± 0.034 vs. 1.037 ± 0.020). After adjusting for baseline differences using ANCOVA, no changes were detected for the right hip after an 8 month intervention. Similar to our results, a cross-sectional study conducted by Sweesy-Barger (127) measured BMD in a group of postmenopausal women who regularly participated in Yoga for a minimum of 3 years, at least twice a week in sessions lasting 60 minutes or longer to a group of age-matched non-Yoga participants performing less than 2 hours of physical activity per week over the past 3 years. These results showed no significant differences in BMD at the spine, femur, or total body. Interestingly, they did not clarify what type of Yoga participants had done in the past 3 years. There are many different types of Yoga to practice in terms of breathing exercise, postures, and spirituality. Depending on different types of Yoga, beneficial effects on bone would vary. The most common style of Yoga in the West is Hatha Yoga, a slow-paced stretching. On the other hand, Ashtanga Yoga is vigorous and highly athletic style, marked by a set sequence of poses (52). In the present study, the 8 month Yoga program was designed to improve BMD following traditional Ashtanga Yoga style. Even though the high intensity of Yoga was performed over the 8 months of training, BMD at each site was not

increased. In previous cross-sectional studies, the results showed higher BMD in women who perform weight-bearing or strength-training exercise compared to normally active controls (28, 62) . We speculate that twice a week high intensity Ashtanga Yoga for 8 months may not impose enough mechanical load on bone for increasing BMD. In addition, the lengths of the training programs for beneficial effects on bone in pre- and postmenopausal women have ranged from 12 to 24 months (10, 11, 79). Therefore, it is also possible that the duration of the 8 month Yoga training in the current study may not be sufficient to find differences in BMD at each site.

Unlike Yoga, results of Tai Chi studies reported that long-term postmenopausal Tai Chi practitioners have higher BMD than age-matched sedentary controls, and have slower rates of bone loss (43, 97, 98, 148, 151). In one cross-sectional study of postmenopausal women, Qin et al. (98) found that subjects in the Tai Chi had significantly higher BMD in the lumbar spine (7.1%), the greater trochanter (7.2%), and Ward's area (7.1%) of the proximal femur. In contrast with previous results, 12 months of regular Tai Chi training did not increase BMD of the spine or femur in postmenopausal women as measured with DXA (21). These findings are similar to my outcomes. There are differences in the study populations (pre- or postmenopausal women), the type, length and intensity of the exercise programs. It is assumed that both slow paced-dynamic movements may not be as effective as weight-bearing or strength exercise for improving BMD in pre- and postmenopausal women.

Most exercise interventions aimed at improving bone health in women have been general rather than specific to either the hip or spine. In the current study, I did not find any significant increases in BMD at the hip even though jumping was

performed as part of the protocol for 8 months. The Triangle posture had two dynamic jumps vertically and subjects performed the Triangle posture every session for entire 8 month intervention. In addition, various balance postures such as tree, eagle, and warrior III postures put weight on weight bearing joints such as the hip. My hypothesis regarding the possibility of increasing the hip BMD by performing weight-bearing balance postures were not supported by the findings of the present study. In contrast, the limited exercise programs specific to the hip or spine have shown that jumping training increased hip, but not spine BMD (10, 11). In an 18 month study of 56 premenopausal women comparing twice weekly strength training to a non-exercising control group, Lohman et al. (79) reported a significant aBMD increase of 2.3% in the lumbar spine and 1.8% in the femoral trochanter and a non significant increase of 1.4% in the femoral neck in the strength training group relative to changes in the control group. In our study, there were no significant group differences in percent changes for the total body, lumbar spine, total hip, femoral neck, and trochanter after the 8 months.

The baseline BMD values at the radius 33% for each group were not significantly different. After the 8 month intervention period, a significant group time interaction was detected as BMD at the radius 33% in YE slightly decreased while CON group slightly increased ($p<0.05$). Paired samples t-tests as post hoc was unable to determine time differences within each group, however, there was a trend ($p=0.061$), indicating that BMD at the radius 33% in YE slightly decreased. The same qualified DXA technician performed all forearm scans at pre and after 8 months and the precision (% coefficient of variation) for the radius 33% is 1.72%. Therefore, the percent changes from baseline (-1.5%, YE vs. 0.87%, CON) were within the precision of the measurement.

Bone Geometry Characteristics

Peripheral Quantitative Computed Tomography (pQCT) has increasingly been utilized for quantitative assessment of the skeleton in adults and children. Unlike DXA, pQCT can provide a 3D assessment of volumetric BMD (vBMD, g/cm^3) and discriminate between trabecular and cortical components of bone, which are known to show different responses to aging, various diseases, and treatments (38, 111). Also, the stress-strain index (SSI), bone strength, can be determined from a cross-sectional geometry and vBMD measured by pQCT. A systematic review of pQCT studies has indicated that the most substantial changes in bone mass and geometry were reported in response to high impact loading activities like volleyball and jumping (3, 135), whereas the smallest effects were observed with low-impact activities like Tai Chi (21). In the present study, we found no significant changes in bone content, bone area, cortical thickness, periosteal circumference, and SSI for total, cortical and trabecular at the tibia 4% and 38%. A previous 5 year follow-up study of pre- and postmenopausal women showed that an age-related decline occurred in the volumetric density of both trabecular and cortical bone. The decline in trabecular density was similar in both age groups, while the decline in cortical density was clearly greater among the postmenopausal women (136). My results showed no decreases in trabecular and cortical density at the tibia 4 and 38% in both YE and CON groups. However, two-way mixed factorial ANOVA with repeated measures revealed a significant time effect ($p<0.01$) for cortical vBMD at the tibia 66% site as both groups significantly decreased after 8 months of intervention (-0.3 ± 0.1, YE vs. -0.1 ± 0.1, CON). However, the coefficient of variation of the pQCT technician for the cortical vBMD is 0.50% so these results are not substantial changes. Overall, the 8 month Yoga program did not increase vBMD at the tibia sites but it remained unchanged in

both groups. On the other hand, Qin et al. (97) found that postmenopausal women who did regular Tai Chi over 4 years had greater vBMD compared to age matched controls. The significant differences found in the Tai Chi group included an approximately 14% higher vBMD in trabecular compartment of the ultradistal tibia.

At baseline, a significant group difference ($p<0.001$) was detected in the total vBMD at the radius 4% as the YE group had a significantly higher value compared to the CON group (360.6 ± 15.4, YE vs. 288.5 ± 33.7). After adjusting baseline differences using one-way ANCOVA, we did not find any significant changes at the radius 4% except for the trabecular vBMD which showed both groups significantly decreased ($p<0.05$). The trabecular vBMD in percent changes is -4.0 ± 2.4 for YE group and $-2.6\%\pm2.4$ for CON group. The coefficient of variation for the trabecular vBMD at the radius 4% site is 1.72%. Therefore, those changes are substantial. Our 8 month Yoga program had diverse weight- bearing postures for the upper body, especially the wrist. In Yoga, postures such as downward facing dog or plank put body weight on the subject's wrist joint, which may create some pressure on bone for increasing BMD. However, my data did not support this hypothesis.

IGF-I Response

Insulin-like growth factor I (IGF-I) functions as a key anabolic regulator of bone cell activity by decreasing collagen degradation and increasing bone matrix deposition and osteoblastic cell recruitment (149). It is well known that both BMD and serum concentration of IGF-I decrease with age. Liu et al. (78) documented that a decrease of the serum IGF-I level occurs almost 20 years before a significant reduction of BMD, thus making it a potential marker for subsequent bone loss in pre and postmenopausal women. They found that 10.6% of premenopausal women had already developed osteopenia, with a mean age of 38, and their serum levels of IGF-I

were also reduced. Also, Adami et al. (4) found that IGF-I decreases steadily with age even during adult premenopausal life. Consistently, we found decreases in serum IGF-I concentrations in our study, indicating that both group significantly decreased after the 8 months ($p<0.01$). Even though subjects in the YE group performed the 8 month Yoga program compared to subjects who were encouraged to maintain their daily activity level, no group time interaction effects for IGF-I were detected. Unexpectedly, serum IGF-I concentrations decreased more dramatically in the YE group (-17.2±4.3%) than the CON group (-6.2±4.2%). The interpretation of our results remains difficult and it is not clear for this reason.

There are limited studies that report higher IGF-I in physically active versus non-active individuals, but the type of activity (aerobic vs. muscle building) associated with higher IGF-I differs (28, 63). Davee et al. (28) reported that performing muscle building exercise was associated with higher IGF-I levels than was engaging in aerobic exercise. However, Kelly et al. (63) demonstrated a strong relationship between aerobic capacity and IGF-I in mature pre- and postmenopausal women, but no independent relationship between muscle strength and IGF-I. Another study regarding the relationship between IGF-I and muscle and bone (123) demonstrated that gymnasts have higher IGF-I and IGF-I/IGFBP3 than runners and higher IGF-I/IGFBP3 than healthy control women. Based on previous studies, the slow dynamic 8 month Yoga program in the present study might be a low intensity to be considered as an effective exercise for increasing serum IGF-I concentrations.

Body Composition

It has been reported that the level of hydration can alter the validity of DXA derived estimates of body composition when multicomponent models are used. Lohman et al. (80) suggested that estimates of body water as a fraction of fat free

mass fall in the expected range of 71 to 75%. In my study, I measured hydration status using urine specific gravity for body composition testing and all subjects were encouraged to drink water the day before the testing to be well hydrated. Subjects for the urine specific gravity were within the range (1.004 – 1.029).

The current study showed no changes in weight, total fat mass, and %fat. Also, there were no significant differences in percent changes for body composition variables ($p>0.05$) from baseline to the end of training. However, bone free lean body mass (BFLBM) significantly increased in both YE and CON groups after the 8 months. Initially we expected to find decreases in fat mass and increases in muscle mass. Unexpectedly, both groups increased in muscle mass as BFLBM in YE group increased slightly more compared to the CON group ($2.1\pm0.7\%$, YE vs. $1.9\pm0.8\%$, CON). There are various possibilities explaining my outcomes. First, frequency of training in my study may not be enough to decrease fat mass. We trained twice a week, one day apart, 60 minutes Ashtanga Yoga. The intensity of training was gradually increased by the number of sun salutations each month and the Yoga session started with a beginning level and ended with intermediate. Therefore, the exercise intensity for first few months might have been too low to impact either fat mass or BFLBM. Hagins et al. (49) investigated whether a Yoga practice using various postures meets the current recommendations for levels of physical activity required to improve and maintain health and cardiovascular fitness. They reported that the metabolic costs of Yoga averaged across the entire session were similar to walking on a treadmill at 2 mph. As a result of this study, Yoga did not meet the ACSM recommendations. In contrast, Kristal et al. (69) found Yoga practice for four or more years was associated with a 3.1-lb lower weight gain among normal weight

(BMI<25) participants and an 18.5-lb lower weight gain among overweight participants.

Another possible explanation for my results could be the seasonal effect. I started the Yoga intervention program in late October and ended in June. Therefore, overall activity level might be reduced during the winter season. The results of BPAQ current scores revealed that the past 8 month activity scores significantly decreased in both groups compared to the baseline ($p<0.05$). During the 8 month intervention period, subjects in the CON group were encouraged to maintain normal daily lifestyle monitored by the BPAQ current questionnaire at 2 month intervals, which was collected by a self-reporting method. Even though there were no significant differences in BPAQ scores for 8 months, individual physical activity levels in the CON group may affect increases in BFLBM. In addition, even though subjects in both groups were encouraged to maintain their body weight, their daily diet was not monitored for 8 months.

Muscle Strength

The subjects in the YE and CON groups performed one repetition maximum (1RM) testing to determine their upper and lower body muscle strength using isotonic exercises (lat pull down, shoulder press, biceps curl for upper body and leg press, knee extension, knee flexion for lower body). At baseline, there was a significant group difference in shoulder press ($p<0.05$) as the CON group was stronger than the YE group for this exercise. After adjusting baseline mean differences, one-way ANCOVA did not detect group differences ($p>0.05$). Interestingly, however, percent changes for shoulder press showed that the YE group had a significantly greater relative increase compared to the CON group ($7.0\pm10.7\%$, YE vs. $-2.1\pm2.7\%$, CON). This could be explained by the large standard error of the mean and measurement

errors from different trained staff. We tried to have the same testers do the pre and post strength testing, but the testing schedule did not meet for the trained staff. However, they followed the same protocol using the pretesting data. For the lat pull down strength, both groups significantly decreased after the 8 months ($p<0.05$). This result showed that lat pull down strength slightly decreased in YE group (-2.5±3.6%) compared to the CON group (-7.0±2.3%). There was a significant group time interaction effect detected in leg press, which increased in YE group, while it decreased in CON group ($p=0.001$). Paired samples t-tests indicated that leg press strength significantly ($p<0.05$) increased in the YE group, whereas the CON group slightly decreased.

It is interesting to note that various weight-bearing balance and static postures such as Warrior series (warrior I, II, reverse warrior, or side angle) were effective for increasing lower body strength. Similar to our results, Tran et al. (130) measured isokinetic muscular strength and isometric muscular endurance using elbow extension (EE), elbow flexion (EF), knee extension (KE), and knee flexion (KF). After the 8 week Hatha Yoga training, isokinetic muscular strength for EE, EF, and KE increased by 31%, 19%, and 28% ($p<0.05$), respectively, whereas isometric muscular endurance for KF increased 57% ($p<0.01$). Compared to our study, subjects were much younger (18 – 27 years) than our subjects (35 – 50 years) and Yoga sessions were offered four times per week for 8 weeks. But in the current study, subjects performed Yoga only twice a week and isotonic strength assessment method was used to measure muscle strength.

Other previous Yoga investigations that specifically measured isometric muscular strength with the hand dynamometer yielded conflicting results. Blumenthal et al (14) showed no strength changes, whereas Madanmohan et al. (82) reported

significant improvements in handgrip strength resulting from Yoga practice. Overall, depending on subject age ranges, gender, frequency of training, different types of Yoga, and measurements, the results of muscle strength could be different.

CHAPTER V

CONCLUSIONS

The purpose of this study was to examine the effects of an 8 month Yoga exercise program on bone biomarkers, areal bone mineral density (aBMD) for the total body, dual proximal femur, and lumbar spine; and volumetric bone mineral density (vBMD) for the tibia and forearm in premenopausal women. The following research questions were investigated: 1) Will an 8 month Yoga exercise program significantly alter rates of bone formation and/or resorption as measured by the bone markers, Bone ALP and TRAP5b? 2) Will an 8 month Yoga exercise program increase BMD in total body, dual femur, lumbar spine, and forearm sites in premenopausal women compared to the control group? 3) Will an 8 month Yoga exercise program alter bone geometry characteristics measured by pQCT?

Research Hypothesis 1. An 8 month Yoga intervention will result in increases in serum Bone ALP concentrations and decreases in serum TRAP5b concentrations.

Yes, the results of the current study support this hypothesis. A significant group x time interaction was detected in serum Bone ALP ($p<0.01$). Post-hoc tests indicated that the CON group had a significant decrease in serum Bone ALP concentration. There were also significant group differences ($p<0.05$) in percent changes for Bone ALP as the YE group had a significantly greater relative increase compared to the CON group (9.1% vs. -7.1%). However, no significant changes were found in TRAP5b in both groups after the 8 month Yoga training intervention. Also, there were no significant group differences ($p>0.05$) in percent changes for TRAP5b and ratio of Bone ALP to TRAP5b, but there was a trend ($p=0.061$) for TRAP5b, which slightly increased in the YE group while serum TRAP5b decreased in the CON group ($1.0\pm4.6\%$ vs. $-8.3\pm6.5\%$).

Research Hypothesis 2. The Yoga intervention will result in increases in BMD for total body, dual femur, lumbar spine, and forearm in premenopausal women.

No, the findings of the current study do not support this hypothesis. The present study did not find any significant BMD differences in the total body, lumbar spine, and dual femur sites between groups ($p>0.05$). No percent changes for BMD values were detected ($p>0.05$). However, a group x time interaction was detected ($p<0.05$) as the radius 33% in YE slightly decreased while the CON group slightly increased ($p<0.05$).

Research Hypothesis 3. The Yoga intervention will alter tibia and radius bone characteristics, specifically, in total volumetric BMD (vBMD), trabecular vBMD, cortical vBMD, and Strength-Strain Index (SSI).

The results of this study do not support this hypothesis. There were no significant changes in bone content, bone area, cortical thickness, periosteal circumference, and SSI for total, cortical and trabecular at the tibia 4% and 38% ($p>0.05$). However, a significant time effect was detected ($p<0.01$) for cortical vBMD at the tibia 66% site as both groups significantly decreased after the 8 month intervention (-0.3 ± 0.1, YE vs. -0.1 ± 0.1, CON). Since the CV% of the pQCT technician for the cortical vBMD is 0.50%, these results are not substantial changes. For the radius 4%, trabecular vBMD significantly decreased in both groups ($p<0.05$). The trabecular vBMD of the radius 4% in percent changes is -4.0 ± 2.4 for the YE group and $-2.6\%\pm2.4$ for the CON group.

The following research subquestions were investigated: 1) Are there significant changes in resting hormone responses (IGF-I) after an 8 month Yoga exercise program? 2) Are there significant changes in body composition variables (% body fat, fat mass (FM), and bone free lean body mass (BFLBM))? 3) Will an 8 month Yoga exercise program increase muscle strength?

Subhypothesis 1. The Yoga intervention will significantly increase the resting serum concentrations of IGF-I in the Yoga exercise group.

The results of this study do not support this hypothesis. A significant time effect was observed ($p<0.01$) as the serum IGF-I levels decreased in both groups after the 8 month intervention (-17.2±4.3%, YE vs. -6.2±4.2%, CON). However, no group x time interaction was detected between groups ($p>0.05$).

Subhypothesis 2. The Yoga intervention will result in significant decreases in % body fat and FM, and increases in BFLBM.

The results of this study do not support this hypothesis. The finding of the current study showed no changes in weight, total fat mass, and %fat ($p>0.05$). Also, there were no significant differences in percent changes for body composition variables ($p>0.05$) from baseline to the end of training. However, both groups significantly increased ($p<0.01$) in muscle mass as BFLBM in the YE group increased slightly more compared to the CON group (2.1±0.7%, YE vs. 1.9±0.8%, CON).

Subhypothesis 3.The Yoga intervention will increase muscle strength in premenopausal women.

The results of the current study did support this hypothesis. The 8 month Yoga training intervention significantly ($p<0.01$) increased leg press strength in the YE group, whereas the CON group slightly decreased. There were also significant group differences ($p<0.01$) in percent changes for shoulder press as the YE group had

a significantly greater relative increase compared to the CON group (7.0±10.7%, YE vs. -2.1±2.7%, CON). However, after adjusting baseline mean differences for shoulder press, one-way ANCOVA did not detect group differences ($p>0.05$). Also, Two-way mixed factorial ANOVA with repeated measures detected a significant time effect ($p<0.05$) for lat pull down strength, which significantly decreased lat pull down muscle strength in both groups. There were no time or group x time interaction effects were observed for biceps curl, knee extension, and knee flexion ($p>0.05$) after the 8 months.

CLINICAL SIGNIFICANCE

High intensity weight bearing endurance or resistance exercise has beneficial effects on bone health in middle-aged and older people. The finding of this study suggest that the 8 month Ashtanga Yoga was able to generate mechanical loading forces to elicit increases in bone formation as well as increases in lower body muscle strength in premenopausal women. My findings support the suggestion that Yoga could be a beneficial alternative for individuals who cannot participate in traditionally recommended exercises for bone health. However, the current study did not produce increases in BMD or vBMD. Future studies are needed which use an increased frequency or longer duration of Yoga training program to determine its effects on BMD.

FUTURE RESEARCH DIRECTIONS

Most previous studies investigating the possible beneficial effects of Yoga have focused on the potential uses as a therapeutic tool. The present study is the first report of a randomized controlled trial of an intervention for Yoga exercise to determine its effects on bone metabolism and hormone responses. Further studies are needed to consider the following issues: 1) quantifying the intensity of weight-bearing

Yoga postures including sun salutations, and 2) a longer duration with increased frequency of Yoga training program for both pre- and postmenopausal women.

In the current study, the intensity of the 8 month Yoga program was gradually increased by the number of sun salutations, which was the first trial. Increased levels of weight-bearing postures such as warrior III or half moon were performed only for the last 2 months. Future studies should focus on high intensity weigh-bearing postures including sun salutations to determine session intensity during the intervention. The findings of the present showed that regular long-term Ashtanga Yoga could be an alternative exercise to increase bone formation and lower body muscle strength in premenopausal women. However, we did not find any significant differences in aBMD and vBMD. The length of the training programs for beneficial effects on bone pre- and postmenopausal women has ranged from 12 to 24 months. Therefore, it is possible that the duration of the 8 month Yoga training may not have been sufficient to find differences in BMD. A longer duration program with increased Yoga frequency should be conducted to investigate differences in BMD. Future research should also explore glutathione, a marker of oxidative stress to provide scientific evidence for increased bone formation.

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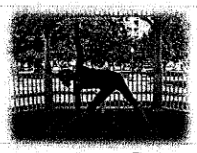
APPENDICES

Appendix A.

Recruitment Flyers, Verbal script, and E-mail script

Female Subjects Needed for YOGA Exercise Intervention Study

Effects of Yoga Exercise on Bone Density and Metabolism in Premenopausal Women



To participate:

- Premenopausal women, age 35-50 years
- Non-smokers
- Not taking hormonal birth control or any medications affecting bone density
- Not engaged in a resistance training or Yoga exercise for at least 12 months
- Weight < 300 lbs
- Women not pregnant

Required Testing: (Pre & Post)

- Bone scans (DXA & pQCT): low-dose radiation will be used to assess bone density
- Blood sampling at Goddard Health Center
- Urine sampling for hydration
- Arterial compliance using PCA
- Muscular strength (1RM)

How long?

Free Yoga classes will be offered:
two times per week (Mondays & Wednesdays, 6:30 ~ 7:30 am) for 8 months at Huston Huffman Center at OU

If you are eligible and interested, please contact:
Sophie Kim at 405-414-1270, sophie74@ou.edu

The University of Oklahoma is an equal opportunity institution.

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Free Yoga Classes

for Yoga Exercise Intervention Study

"Effects of Yoga Exercise on Bone Density and Metabolism in Premenopausal Women"

Bone Density Laboratory
Department Health and Exercise Science
University of Oklahoma

Who is eligible?

-Premenopausal women, age 35-50 years
-Non-smokers
-Women not pregnant
-Not taking hormonal birth control or any medications affecting bone density
-Not engaged in a resistance training or Yoga exercise for at least 12 months

Required Testing (Pre & Post)

-Bone scans (DXA & pQCT): low-dose radiation will be used to assess bone density
-Blood sampling at Goddard Health Center at OU
-Urine sample for hydration
-Arterial compliance using PCA
-Muscular strength (1RM)

How long?

Free Yoga Classes will be offered:
Two times per week (Mondays & Wednesdays, 6:30 ~7:30 am) for 8 months
at Huston Huffman Center at OU
If you are eligible and interested, please contact:

Sophie Kim at 405-414-1270, sophie74@ou.edu

Verbal Recruitment Script

Hello, my name is _____, I am a graduate student in the Department of Health and Exercise Science at the University of Oklahoma. I invite you to participate in a research study we are conducting. The title of the study is "Effects of Yoga Exercise on Bone Density and Bone Metabolism in Premenopausal Women." We are trying to determine the effects of an eight-month Yoga exercise program bone biomarkers (BAP and TRAP5b), BMD of total body, femur, lumbar spine, tibia, and forearm. Also, this research will be performed to determine bone geometry characteristics and hormone responses to the Yoga intervention. The length of this study is about 9 months including the Yoga exercise intervention, baseline, and post-testing.

We are looking for the subjects, who are healthy premenopausal women between the ages of 35 and 50, not taking hormonal birth control, non-smokers, not pregnant, or any medication that affects bone density. Also, they are not engaged in resistance training or in Yoga exercise for at least 12 months. Once you decide to participate in this study, you will be randomly assigned to either a Yoga exercise group (YE), or a control group (CON). Yoga classes will be offered two times per week (MW and 6:30 ~ 7:30 am) for 8 months at the Huston Huffman Center in the University of Oklahoma. Subjects in CON group will not receive the Yoga exercise intervention and they will be encouraged to maintain normal daily lifestyle monitored by the bone-specific physical activity questionnaire at 2 month intervals for 8 months.

Testing will consist of health questionnaires regarding your health history, calcium intake, menstrual history, 3-day dietary log, and bone-specific physical activity in the Bone Density Lab at OU. You will complete 5 DXA and 5 pQCT scans to measure regional bone mineral densities and bone characteristics. A very low level of radiation exposure will be experience from the DXA and pQCT scans. Testing will also include a resting blood sample (about 6 ml; 1.5 teaspoons) to measure two bone markers (TRAP5b and BAP) and two hormones (IGF-I) and a urine sample (2-3oz) for body composition measurements. For Blood vessel function, arterial compliance will be measured using Pulse Contour Analysis. All testing will be performed at baseline and after 8 months.

There is possibility of mild soreness because of the exercise, but should recover within a couple of days. There is also possibility of mild bruising from the blood sampling procedures. The neural stimulation feels like a quick pinch on your skin.

I would be happy to answer any additional questions that you may have about the study. Thank you!

Mass Email Message

Hello,

SoJung Kim, M.S., and Debra Bemben, Ph.D., are conducting a study examining the effects of Yoga exercise on bone density and metabolism in premenopausal women. This study will be conducted at the Bone Density research Laboratory on the University of Oklahoma Norman campus.

The researchers are seeking premenopausal women, age 35 to 50, who are healthy, non-smokers, not pregnant, not taking hormonal birth control, or any medication that affects bone density. Also, they are not engaged in resistance training or in Yoga exercise for at least 12 months. Once you decide to participate in this study, you will be randomly assigned to either a Yoga exercise group (YE), or a control group (CON). Yoga classes will be offered two times per week (MW and 6:30 ~ 7:30 am) for 8 months at the Huston Huffman Center in the University of Oklahoma. Subjects in CON group will not receive the Yoga exercise intervention and they will be encouraged to maintain normal daily lifestyle monitored by the bone-specific physical activity questionnaire at 2 month intervals for 8 months.

Total participation time is eight months, during which, the subjects will be required to give blood and urine samples and receive arterial compliance and bone scans at baseline and post-intervention. Participants in YE group will be required to complete an eight-month Yoga exercise intervention that will meet for one hour, two times per week.

If interested, contact SoJung Kim at 405-414-1270, sophie74@ou.edu for more information. Please include your name, telephone number and the best time to reach you in your message.

"The OU IRB has approved the content of this message but not the method of distribution.

The OU IRB has no authority to approve distribution by mass email."

Appendix B.

Informed Consent, Authorization to Use or Disclose Protected Health Information

University of Oklahoma
Institutional Review Board
Informed Consent to Participate in a Research Study

Project Title: Effects of Yoga Exercise on Bone Density and Bone Metabolism in Premenopausal Women
Principal Investigator: Dr. Debra A. Bemben, PhD, Sojung Kim, Co-PI
Department: Health and Exercise Science

You are being asked to volunteer for this research study. This study is being conducted at the University of Oklahoma. You were selected as a possible participant because you meet the criteria of a healthy premenopausal female participant who is between 35 and 50 years of age, non-smoker, not taking hormonal birth control, not taking any medication which affects bone density, and have not engaged in resistance training or in Yoga exercise for at least 12 months prior to the study.

Please read this form and ask any questions that you may have before agreeing to take part in this study.

Purpose of the Research Study

The purpose of this study is to examine the effects of an eight-month Yoga exercise program on bone health in premenopausal women. Specifically, bone density and blood markers of bone metabolism will be measured before and after the 8 months of Yoga training. Another purpose of this study is to examine the effects of the Yoga intervention on hormones, body composition, blood vessel function, and muscle strength.

Previous studies have shown that Yoga increases in muscular endurance, flexibility, and aerobic fitness, but no studies to date have examined the possible benefits of Yoga training for bone health.

Number of Participants

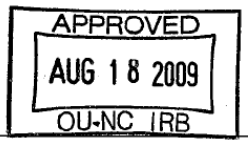
About 70 people will take part in this study.

Procedures

If you agree to be in this study, you will be asked to do the following:

1. Pre-Screening

Pre-Screening will include completion of all questionnaires and the initial assessment of muscle mass and bone densities by Dual Energy X-Ray Absorptiometry (DXA). If there are contraindications for participation as determined from the completed questionnaires or the DXA measures (i.e., low bone mineral densities), you will be excluded from the study.



2. Questionnaires

You will be asked to complete an informed consent and questionnaires that include calcium intake, 3-day dietary log, health history, menstrual history, and bone-specific physical activity.

3. Body Composition Measurement

A refractometer (VEE GEE®, Model CLX-1) will be used to measure urine specific gravity for hydration status at baseline and after 8 months of training. If you have urine specific gravities out of ranges (1.004 ~ 1.029), you will not be allowed to undergo the body composition assessment. If you fail the hydration test, you will be assessed again. Dual Energy X-Ray Absorptiometry (DXA; GE Lunar Prodigy) will be used to measure body composition of the whole body in the Bone Density Laboratory by a trained DXA technician under the supervision of Dr. Debra Bemben.

4. Dual Energy X-Ray Absorptiometry (DXA)

A series of bone scans will be performed by Dual Energy X-Ray Absorptiometry (DXA) at the Bone Density Laboratory in the Department of Health and Exercise Science at baseline and after 8 months. The test during each visit will include five scans (total body; lumbar spine; dual proximal femur right & left; and forearm) which will take about 25 ~ 30 minutes to complete. The DXA scan results in x-ray exposure that is similar to the radiation exposure Americans receive in several days from natural background radiation (being in the sun and radioactivity in the soil). You will complete a total of 10 bone scans: 5 scans at each visit at baseline and after 8 months of training.

5. peripheral Quantitative Computed Tomography (pQCT)

A series of peripheral Quantitative Computed Tomography (pQCT) scans will be performed during each visit in the Bone Density Laboratory. pQCT is a type of x-ray procedure, and you will have 5 pQCT scans done each visit. Scans will be obtained on the non-dominant leg at 4%, 38%, and 66% and forearm at 4% and 66% of their lengths, respectively. This will take approximately 25 ~ 30 minutes to complete. These scans are non-invasive and only require that you sit still for the test to be completed. You will complete a total of 10 bone scans: 5 scans at each visit at baseline and after 8 months of training.

6. Blood Sampling and Parameters of Interest

You will be asked to visit Goddard Health Center before the study begins and at the end of the intervention (8 months) so that resting blood samples can be obtained by a nurse in the morning following an 8-hour overnight fast during each of the 2 visits from venapuncture in a forearm vein (approximately 6 ml, or about 1.5 teaspoons). These samples will be used to measure two biochemical markers of bone metabolism (C-Telopeptide of Type I Collagen and Bone-Specific Alkaline Phosphatase) and two hormones (Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein 3). The safety of precautions will be used including cleaning of the venipuncture site with alcohol, use of new sterile disposable needles/syringes and changing of disposable gloves in between subjects by the registered nurse/phlebotomist. Blood samples will be

kept for 2 years after the results have been published in case samples have to be reanalyzed. The subjects do NOT have to pay for this procedure.

7. Arterial Compliance

Non-invasive measures of arterial compliance (at the wrist) will be assessed by the HDI/Pulsewave CR-2000 (Hypertension Diagnostics, Eagon MN) in the morning following 8-hour overnight fast at baseline and after 8 months. You will be in a supine position and an upper arm blood pressure cuff will be place on you left arm and an initial blood pressure measurement will be obtained. Your right wrist will be placed in a wrist stabilizer to gently immobilize the wrist and stabilize the radial artery making it accessible for placement of an arterial pulse pressure sensor place over the radial artery. Pulse pressure waveforms will then recorded and calibrated by the oscilloscope method for 30 seconds.

8. 1RM Strength Testing

You will have your muscle strength of the lower body (leg press, knee extension, and knee flexion) and upper body (lat pull down, shoulder press, and biceps curl) assessed by one-repetition maximum (IRM) tests by trained testers. You will warm-up for 5-10 minutes on a stationary bicycle followed by stretching. Following one minute rest period you will become familiar with each of the resistance machines by performing 8-10 repetitions of a light load (~50% of predicted 1RM). After one minute rest, you will lift a load (~80% of estimated 1RM) though the full range of motion. After each successful lift, the weight will be increased until a failed attempt occurs. One minute rests are given between each attempt and the 1RM is determined within 5 attempts and 5 minutes rest will separate the individual tests. Your strength will be assessed at the beginning of the study and at the end of the intervention (8 months).

9. Specific Exercise Protocols for the Yoga Exercise Sessions

You will be randomly assigned either to a Yoga exercise group (YE, n=35) or a control group (CON, n=35). Yoga classes will be offered two times per week, one day apart between sessions for 8 months and subjects will take morning sessions (6:30 ~ 7:30 am, MW) at the Huston Huffman Center in the University of Oklahoma. Subjects in CON group will not receive the Yoga exercise intervention and they will be encouraged to maintain normal daily lifestyle monitored by the bone-specific physical activity questionnaire at 2 month intervals for 8 months. Each Yoga session will consist of 15 minutes of warm-up exercises, 40 minutes of asanas (Yoga postures) and 5 minutes of cool-down with relaxation (the corpse pose). Subjects in the Yoga exercise group will record their Heart Rate using the palpation method (radial artery) and the Rating of Perceived Exertion (RPE) at the beginning of the Yoga exercise, after sun salutations, and before cool-down. During the first 5 minutes of warm-up, subjects will be instructed to breathe the most common yogic breathing technique, Ujjayi breathing, and they will encouraged to breathe the Ujjayi breathing technique throughout the sessions. Dynamic and static stretching will be introduced during the warm-up at the beginning with either sitting, supine, or standing postures. A certified Yoga instructor will lead all Yoga sessions and precisely teach Yoga postures with the same manner of instructions. The modified postures will be instructed to subjects who may not follow the same directions.

Subjects will record whether they do the modified postures after Yoga sessions. Subjects will be encouraged to perform all asanas as accurately as possible. Each posture will be performed in both directions (right and left) and the Yoga session routines will be performed in the same order. Forty- minutes of asanas will consist of the sun salutation (I, II), standing, balancing, sitting, and supine postures. During the first 4 months, the sun salutation I with a triangle pose and warrior series will be instructed and jumping will be progressively included. The sun salutation II involved in jumping will be performed at the last 4 months. The number of sun salutation I and II performed with jumping will be increased in each month. Subjects will be asked to wear appropriate clothing (e.g., shorts and sweat pants) with at least one hour fasted status and be informed to know pre-considerations for Yoga exercise.

Length of Participation

There are 4 visits to the University of Oklahoma Bone Density Laboratory. These visits will occur prior to training and at the end of training (8 months). Each of these visits will take approximately 1 hour and 30 minutes to complete.

This study has the following risks:

1. This research study involves exposure to radiation from a total of 10 DXA and 10 pQCT scans over 2 visits which are types of x-ray procedure. This radiation exposure is not necessary for medical care and is for research purposes only. You will receive radiation exposure from all 10 DXA scans and 10 pQCT of less than 5 % of the normal amount of natural background radiation (~300 mrem/year). Although the amount of radiation exposure received in the study is minimal, it is important for the subject to be aware that the risk from radiation exposure is cumulative over a lifetime. If after these measures it appears as though you have abnormally low bone mineral densities in the hips or spine (osteoporosis), you will be asked to end your participation in this study and encouraged to visit your physician.

2. There is a possibility of temporary muscle soreness occurring 24-48 hours after the 1RM testing. You may also experience temporary muscle soreness that is experienced when beginning a new Yoga exercise. Although trained personnel will supervise both exercise sessions, there is a possibility of an orthopedic or muscle injury.

3. Blood draws will be performed by qualified personnel, but there may be possible discomfort at the site of the venipuncture and possible bruising during and after your blood draws. Blood samples will be kept for 2 years after the data has been published in case samples have to be reanalyzed. Thereafter the blood will be discarded in an approved biohazard waste receptacle. Blood samples will be taken at baseline and after 8 months. These samples will be used to measure two biochemical markers of bone metabolism (C-Telopeptide of Type I Collagen and Bone-Specific Alkaline Phosphatase) and two hormones (Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein 3). These assays will be conducted after all the subjects have completed the study, since it is critical that all samples for a subject be run within the same assay. This procedure is necessary to reduce the technical error associated with the assays and it is considered standard scientific procedure in the bone metabolism literature. Therefore, it may be 1 to 1.5 years before the blood results can be made available to subjects. It should be noted that all of the procedures in this study are non-

diagnostic and will be used for research purposes only. We expect that some subjects may have low BMD or abnormal blood test results. The following procedures will be followed at the end of the study should Dr. Debra Bemben (PI) or other members of the research team observe that a subject has an abnormal value for the blood tests or bone scans: 1) the subject will be contacted by telephone or email to come to the lab to receive a copy of her results; and 2) the investigators named above will recommend that the subject bring the results to her personal physician for consultation.

"Pregnant women should not be exposed to radiation. Therefore, I verify that I am not pregnant (if participant is uncertain about pregnancy, then she should not perform this test). Radiation safety levels for fetuses have not been established and therefore no additional exposure whatsoever can be considered safe."

I verify that I have read and understood the above radiation warning.
Initials: _____

Benefits of being in the study are

1. Information regarding your bone scans and body composition results will be provided at the beginning and at the end of the study upon your request for interpretation by your physician.

2. Subjects in the Yoga exercise group will be given free Yoga sessions for 8 months.

3. Participants in this study will contribute to science because the information obtained will help us gain insight how Yoga exercise affects bone health.

Injury

In case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company may be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Confidentiality

In published reports, there will be no information included that will make it possible to identify you without your permission. Research records will be stored securely and only approved researchers will have access to the records.

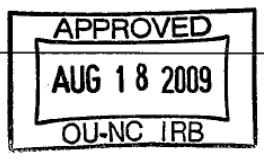
There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the OU Institutional Review Board.

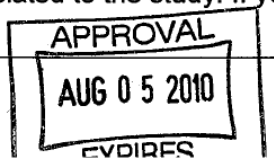
Compensation

You will not be reimbursed for your time and participation in this study.

Voluntary Nature of the Study

Participation in this study is voluntary. If you withdraw or decline participation, you will not be penalized or lose benefits or services unrelated to the study. If you decide to





participate, you may decline to answer any question and may choose to withdraw at any time.

Video Recording of Study Activities

To assist with accurate recording of your responses, interviews may be recorded on a video recording device. You have the right to refuse to allow such recording. Please select one of the following options:

I consent to video recording. ___ Yes ___ No.

Photographing of Study Participants/Activities

In order to preserve an image related to the research, photographs may be taken of participants. You have the right to refuse to allow photographs to be taken without penalty. Please select one of the following options.

I consent to photographs. ___ Yes ___ No.

Contacts and Questions

If you have concerns or complaints about the research, the researcher(s) conducting this study can be contacted at 405-325-5211 or via email: dbemben@ou.edu for Dr. Debra Bemben and sophie74@ou.edu for Sojung Kim.

Contact the researcher(s) if you have questions or if you have experienced a research-related injury.

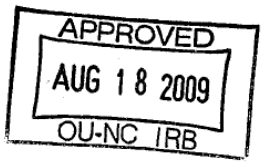
If you have any questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than individuals on the research team or if you cannot reach the research team, you may contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu.

You will be given a copy of this information to keep for your records. If you are not given a copy of this consent form, please request one.

Statement of Consent

I have read the above information. I have asked questions and have received satisfactory answers. I consent to participate in the study.

Signature Date



UNIVERSITY OF OKLAHOMA – NORMAN CAMPUS
INSTITUTIONAL REVIEW BOARD

AUTHORIZATION TO USE or DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.

Title or Research Project: Effects of Yoga Exercise on Bone Density and Bone Metabolism in Premenopausal Women

Principal Investigator: Dr. Debra A. Bemben, PhD, and Sojung Kim, Co-PI

IRB Number:

Address: 1401 Asp Ave. Norman, OK 73019

Phone Number: 405-325-5211

If you decide to join this research project, University of Oklahoma (OU) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private information To be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to analyze the data from the project and present the information in aggregate form.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research

APPROVED
AUG 18 2009
OU-NC IRB

AUG 05 2010

sponsor, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services (HHS). The researchers may also share your private information with all researchers collaborating on this project.

Confidentiality. Although the research may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

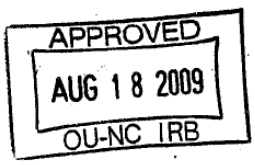
Voluntary Choice. The choice to give OU researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

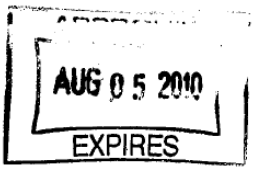
Refusing to give permission will not affect your ability to get routine treatment or health care from OU.

Revoking Permission. If you give OU researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OU researchers to use or share your private information for their research will end when all data from the project has been analyzed and all reports have been published. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma
1000 Stanton L. Young Blvd., STE 221,
Oklahoma City, OK 73117
If you have questions, call: (405) 271-2511





Giving Permission. By signing this form, you give OU and OU's researchers led by Dr. Debra A. Bemben, PhD, permission to share your private information for the research project called Effects of Yoga Exercise on Bone Density and Bone Metabolism in Premenopausal Women.

Subject Name:

_____ _____

Signature of Subject Date
Or parent if Subject is a Child

Or

_____ _____

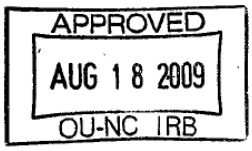
Signature of Legal Representative** Date

**If signed by a legal Representative of the Subject, provide a description of the relationship to the Subject and the Authority to Act as Legal Representative:

_____

OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.



Appendix C.

Calcium Intake, 3-day Dietary Log, Health History, Menstrual History and Bone-specific Physical Activity Questionnaires

BONE DENSITY LABORATORY
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
UNIVERSITY OF OKLAHOMA

CALCIUM INTAKE ESTIMATION

NAME_____ TODAY'S DATE_____

Complete this form (where indicated) to represent your dietary intake in the past year.

				I EAT THIS FOOD:	
				EVERY WEEK	EVERY DAY
Tally *(office use only)*	**Score** *(office use only)*	**Food Type**	**Serving Size**	**Write in # servings/week**	**Write in # servings/day**
	300	Milk - whole, w%, skim	1 cup		
	150	Cheese food or spread	1 oz		
	150	Cheese sauce	1/4 cup		
	150	American cheese	1 slice		
	150	Cottage cheese	1 cup		
	250	Ricotta cheese	1 oz		
	150	Blue cheese	½ cup		
	200	Natural cheese (except cream cheese) includes cheddar, Swiss, mozzarella, and so forth	1 oz		
	285	Buttermilk	1 cup		
	300	Yogurt, flavored or plain	1 cup		
	450	Fast food milkshake	12 oz		
	165	Cocoa from mix	1 packet		
	330	Eggnog	1 cup		
	280	Chocolate milk	1 cup		
	250	Macaroni and cheese, cheese souffle, lasagna, quiche, cannelloni, pizza	1 serving		
	180	Cream soup or chowder with milk	1 cup		
	115	Almonds	1/3 cup		
	180	Broccoli	1 cup		
	85	Beet greens, spinich	½ cup		
	160	Baked beans	1 cup		
	100	Figs	5 dried		
	140	Scalloped potatoes	1 cup		
	150	Soybeans	1 cup		
	150	Tofu	½ cup		

PLEASE TURN OVER

				I EAT THIS FOOD: EVERY WEEK	EVERY DAY
Tally *(office use only)*	Score *(office use only)*	**Food Type**	Serving Size	Write in # servings/week	Write in # servings/day
	30	Bread, white or whole grain	1 slice		
	120	Waffle or pancake	1 large		
	50	Muffin, biscuit, cornbread	1 medium		
	40	Rolls, buns	½		
	225	Egg McMuffin	1		
	130	Fast food cheeseburger or hamburger	1		
	110	Enchilada or bean burrito	1		
	125	Creamed fish and meats	1 cup		
	130	Shellfish, cooked	4 oz		
	200	Canned salmon with bones	½ cup		
	200	Sardines, smelts, herring	½ cup		
	100	Fudgesicle	1		
	125	Custard pie	1 slice		
	175	Ice cream or ice milk	1 cup		
	190	Pudding with milk	½ cup		
	200	Frozen yogurt	1 cup		

Please list any dietary supplements (single and multi-vitamins, calcium, herbal, etc.) you take below, including the brand name and amount (mg).

1. _____
2. _____
3. _____
4. _____
5. _____

Bone Density Research Laboratory
OU Department of Health and Exercise Science
3-Day Dietary Log

Subject ID_____
Date_____

Instruction:

Please record everything that you eat for **two days during the week** and **one day during the weekend**. Include the food/drink item with brand names if applicable, the amount ingested, and method of preparation, if applicable. Please be as specific as possible.

Serving Size Handy Guide: See Attached Appendix

Day 1 during the week: _____

Meal/Time	Food/Drink	Amount (1 cup, 8 oz, number of slices, etc.)	How Prepared (fried, baked, etc.)
Breakfast			
Snack			
Lunch			
Snack			
Dinner			
Snack			

Bone Density Research Laboratory
OU Department of Health and Exercise Science
Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.
(NOTE: The following codes are for office use only: RF; MC; SLA; SEP)

Part 1. Information about the individual

1. _____
Date

2. _____ _____
Legal name Nickname

3. _____
Mailing address

_____ _____
Home phone Business phone

4. Gender (circle one): Female Male (RF)

5. Year of birth: _____ Age _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

(SLA) More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8. Date of: Last medical physical exam: _____ Last physical fitness test: _____
Year Year

9. Circle operations you have had:

Back (SLA)	Heart (MC)	Kidney (SLA)	Eyes (SLA)	Joint (SLA)	Neck (SLA)
Ears (SLA)	Hernia (SLA)	Lung (SLA)	Other _____		

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism (SEP)	Diabetes (SEP)	Kidney problem (MC)
Anemia, sickle cell (SEP)	Emphysema (SEP)	Mental illness (SEP)
Anemia, other (SEP)	Epilepsy (SEP)	Neck strain (SLA)
Asthma (SEP)	Eye problems (SLA)	Obesity (RF)
Back strain (SLA)	Gout (SLA)	Osteoporosis
Bleeding trait (SEP)	Hearing loss (SLA)	Phlebitis (MC)
Bronchitis, chronic (SEP)	Heart problems (SLA)	Rheumatoid arthritis (SLA)
Cancer (SEP)	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia (SEP)	Thyroid problem (SEP)
Concussion (MC)	Hyperlipidemia (RF)	Ulcer (SEP)
Congenital defect (SEP)	Infectious mononucleosis (MC)	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner (MC)	Epilepsy medication (SEP)	Nitroglycerin (MC)
Diabetic pill (SEP)	Heart-rhythm medication (MC)	Estrogen
Digitalis (MC)	High-blood-pressure medication (MC)	Thyroid
Diuretic (MC)	Insulin (MC)	Corticosteroids
Asthma	Other _____	

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood (MC)
1 2 3 4 5

b. Abdominal pain (MC)
1 2 3 4 5

c. Low back pain (SLA)
1 2 3 4 5

d. Leg pain (MC)
1 2 3 4 5

e. Arm or shoulder pain (MC)
1 2 3 4 5

f. Chest pain (RF) (MC)
1 2 3 4 5

g. Swollen joints (MC)
1 2 3 4 5

h. Feel faint (MC)
1 2 3 4 5

I. Dizziness (MC)
1 2 3 4 5

j. Breathless with slight exertion (MC)
1 2 3 4 5

Part 3. Health-related behavior

13. (RF) Do you now smoke? Yes No

14. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled		Less than 5, none inhaled	

15. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

16. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

17. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

18. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

19. Do you exercise or labor at least three times a week?

1. Yes 2. No

20. Have you been exposed to X-Rays/radiation for research and/or medical purposes within the previous 12 months?

    1. Yes 2. No

If yes, please indicate the type and number of X-Ray/radiation exposures in the previous year.

Chest X-Ray _____ (number) Dental X-Ray _____ (number)

Mammograms _____ (number) DXA Scans _____ (number)

CT scans _____ (number) pQCT Scans _____ (number)

Other _____ (number)

Bone Density Research Laboratory
Department of Health and Exercise Science
University of Oklahoma

MENSTRUAL HISTORY QUESTIONNAIRE

Subject ID: _____ Date: _____

We are asking you to give us as complete a menstrual history as possible. All information you provide will be strictly confidential.

Are you pregnant? (circle your response below)

YES – Do not complete the rest of this form.

NO – Complete sections A and B of this form.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?

2. Circle the months in which your period occurred. This means from this time last year until the present month.

   JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC

3. What is the usual length of your menstrual cycle (first day menses to first day next menses)

   ____ days. Today is day _____ of your present menstrual cycle.

4. What was the date of your last period?

5. When do you expect your next menstrual period?

6. What is the length (number of days) of your menstrual flow on the average?

SECTION B: PAST MENSTRUAL HISTORY

1. At what age did you experience your **first** menstrual period?

2. Were your periods regular (occurring monthly) during the first two years after menstruation began? If no, at what age did your periods eventually become regular?

3. Did you perform any form of athletic training prior to your first menstrual period? If yes, indicate type of training (i.e., gymnastics, track, basketball, etc.) and the number of years you trained for each activity.

4. Has there been any time in the past where your periods were irregular or absent? If no, skip to question 5.

   If yes, did these periods coincide with unusual bouts of training, or with a period of stress?
   How long did this occur?

5. Have you ever consulted a doctor about menstrual problems (specifically, about irregular or missing periods)? If no, skip to question 6.

   If yes, have you ever been diagnosed as having a shortened luteal phase?

   Have you ever been tested to determine if you were ovulating normally?

6. Have you ever consulted a physician about any problems relating to your hormonal system? If so, please explain.

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

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

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

Age: Activities	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

2. Pease list the sports or other physical activities (be as specific as possible) you participated in regularly during the last 12 months and indicate the average frequency (sessions per week)?

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

BONE-SPECIFIC PHYSICAL ACTIVITY QUESTIONNAIRE
Developed by B.K. Weeks and B.R. Beck
Griffith University, QLD, Australia

Appendix D.

IRB Approval Letter

The University of Oklahoma

OFFICE FOR HUMAN RESEARCH PARTICIPANT PROTECTION

IRB Number: 12669
Meeting Date: August 06, 2009
Approval Date: August 18, 2009

August 19, 2009

Debra Bemben, Ph.D.
Health & Exercise Science
1401 Asp Avenue, HHC 104
Norman, OK 73019

RE: Effects of Yoga Exercise on Bone Density and Bone Metabolism in Premenopausal Women

Dear Dr. Bemben:

The University of Oklahoma Norman Campus Institutional Review Board (IRB) reviewed the above-referenced research protocol at its regularly scheduled meeting on August 06, 2009. It is the IRB's judgement that the rights and welfare of the individuals who may be asked to participate in this study will be respected; that the proposed research, including the process of obtaining informed consent, will be conducted in a manner consistent with the requirements of 45 CFR 46, as amended; and that the potential benefits to participants and to others warrant the risks participants may choose to incur.

On behalf of the IRB, I have verified that the specific changes requested by the convened IRB have been made. Therefore, on behalf of the Board, I have granted final approval for this study.

This letter documents approval to conduct the research as described:

Consent form - Subject Dated: July 27, 2009
Protocol Dated: July 27, 2009
Priv - Research Auth 1 Dated: July 27, 2009
Other Dated: July 27, 2009 Journal Article
Survey Instrument Dated: July 27, 2009 Bone Density Lab - Yoga Exercise Log
Survey Instrument Dated: July 27, 2009 Bone Density Lab - Health Status Questionnaire
Survey Instrument Dated: July 27, 2009 Bone Desity Lab - 3 Day Dietary Log
Survey Instrument Dated: July 27, 2009 Bone Density Lab - Menstrual History Questionnaire
Survey Instrument Dated: July 27, 2009 Bone Density Lab -Calcium Intake Estimation Log
Consent form - Other Dated: July 27, 2009 Bone Density Laboratory -Individual's Consent Form
Survey Instrument Dated: July 27, 2009 Bone Density Lab - Heart Rate & RPE Records
Recruitment flyer Dated: July 27, 2009
Other Dated: July 27, 2009 Verbal Recruitment Script
Other Dated: July 27, 2009 Mass Email Message
Other Dated: July 27, 2009 Pre-Considerations for Yoga Exercise
Consent form - Subject Dated: July 29, 2009 Revised
IRB Application Dated: August 13, 2009 Revised
Other Dated: August 13, 2009 Apdx B -Student as PI Faculty Sponsor's Assurance

As principal investigator of this protocol, it is your responsibility to make sure that this study is conducted as approved by the IRB. Any modifications to the protocol or consent form, initiated by you or by the sponsor, will require prior approval, which you may request by completing a protocol modification form.

The approval granted expires on August 05, 2010. Should you wish to maintain this protocol in an active status beyond that date, you will need to provide the IRB with an IRB Application for Continuing Review (Progress Report) summarizing study results to date. The IRB will request a progress report from you approximately two months before the anniversary date of your current approval.

If you have questions about these procedures, or need any additional assistance from the IRB, please call the IRB office at (405) 325-8110 or send an email to irb@ou.edu.

Cordially,

Lynn Davenport, Ph.D.
Chair, Institutional Review Board

1300 Asp Avenue, Suite 316, Norman, Oklahoma 73019-3085 PHONE: (405) 325-8110 FAX:(405) 325-2373

APPENDIX E.

1RM and HR Logs

1-RM PRE-TESTING

Bone Density Research Laboratory

Subject (ID): _____ Date: _____

Tester: _____ Testing-Time: _____

☺Warm-Up for 5-10 minutes on a stationary bicycle

		Seat Adjustments	Warm-Up ~50% of predicted 1RM **(# of plates)**	Start with ~80% of estimated 1RM **# of plates (5 trials)**					**Final 1RM in Kg**
				1	**2**	**3**	**4**	**5**	
Upper Body	**Lat Pull Down**								
	Shoulder Press								
	Biceps Curl								
Lower Body	**Leg Press**								
	Knee Extension								
	Knee Flexion								

Heart Rate (HR) and Rate of Perceived Exertion (RPE) Records

Bone Density Research Laboratory

OU Department of Health and Exercise Science

Subject ID: _____

Month 2010	Dates	HR/ RPE	Resting	After SS	Before corpse
April	W 21	HR			
		RPE			
	W 28	HR			
		RPE			
May	W 5	HR			
		RPE			
	W 12	HR			
		RPE			
	W 19	HR			
		RPE			
	W 26	HR			
		RPE			
June	W 2	HR			
		RPE			
	W 9	HR			
		RPE			
	W 16	HR			
		RPE			

1) HR: Place your index and middle fingers together on the opposite wrist, about 1/2 inch on the inside of the joint, in line with the index finger, then count the number of beats for 15 seconds.

2) RPE Scale

6	No exertion at all
7	Extremely light exertion
8	
9	Very light exertion
10	
11	Light exertion
12	
13	Somewhat hard exertion
14	
15	Hard (heavy) exertion
16	
17	Very hard exertion
18	
19	Extremely hard exertion
20	Maximal exertion

Appendix F.

An 8 Month Yoga Program

The Yoga Warm-up

Sitting Warm-up	Standing Warm-up
Easy pose with breathing	Mountain pose with breathing
Neck stretching	Neck stretching
Chest, Back, Side using the arms	Chest, Back, Side using the arms (x4)
Twist torso	Twist torso
Arm stretching	Shoulder stretching
Staff pose	Arm stretching
Foot stretching	Side angle squat (x10)
Side stretching	Squatting leg-out adductor stretching
Revolved head to knee pose (variations I, II)	High lunge (lift hips up & down x10)
One-legged king pigeon pose (variation)	Front-back hand clapping (x10)
Butterfly pose	Up-down swing the arms (x10)
Front-back hand clapping (x10)	
Up-down swing the arms (x10)	
Supine Warm-up	**Sitting & Standing Warm-up**
Supine pose with knee bended	Easy pose with breathing
Neck stretching	Neck stretching
Wind removing pose	Chest, Back, Side using the arms
Revolved abdomen pose	Twist torso
Double knee hugs pose	Arm stretching
Staff pose	Butterfly pose
Upward plank pose	Side angle squat (x10)
Seated forward bend pose	Squatting leg-out adductor stretching
Half lord of the fishes pose	High lunge pose (lift hips up & down x10)
Rolling like a ball (x5, Advanced x5)	Front-back hand clapping (x10)
Table pose	Up-down swing the arms (x10)
Cat & Cow pose	
Spinal balance pose	
Child pose	
Downward-facing dog pose	
One leg forward-backward steps (x10)	
Front-back hand clapping (x10)	
Cool down	
Wind removing pose (Left & Right)	
Revolved abdomen pose	
Double knee hugs pose	
Corpse pose	
Side lying pose	
Easy pose with breathing	

The Sun Salutation (SS) Yoga Routines

SS I	SS II
Mountain pose	Mountain pose
Prayer pose	Prayer pose
Upward hand pose	Chair pose
Standing forward bend pose	Standing forward bend pose
Plank pose	Half stand forward bend pose
Four-limbed staff pose	Plank pose
Upward-facing dog pose	Four-limbed staff pose
Downward-facing dog pose	Upward-facing dog pose
Standing forward bend pose	Downward-facing dog pose
Upward hand pose	Warrior I (right)
Prayer pose	Warrior II
	Reverse warrior pose
	Side angle pose
	Plank pose
	Four-limbed staff pose
	Upward-facing dog pose
	Downward-facing dog pose
	Warrior I (left)
	Warrior II
	Reverse warrior pose
	Side angle pose
	Plank pose
	Four-limbed staff pose
	Upward-facing dog pose
	Downward-facing dog pose
	Half standing forward bend pose
	Standing forward bend pose
	Chair pose
	Prayer pose

The number of Sun Salutations (I, II) performed with jumping for 8 months

Months	1	2	3	4	5	6	7	8
SS I	3	4	5	6				
SS II					4	5	6	7

Yoga routines for 8 months

Months	Mondays	Wednesdays
1	Sitting warm-up routine SS I 3 (no jump involved) Triangle pose (x2) Warrior II Reverse warrior pose Side angle pose Extended side angle pose Wide legged forward bend pose Single-leg balance pose Tree pose Side plank pose Bridge pose Double legs lift Cool down	Sitting warm-up routine SS I 3 (no jump involved) Triangle pose (x2) Warrior II Reverse warrior pose Side angle pose Extended side angle pose Wide legged forward bend pose Single-leg balance pose Tree pose Wide angle pose Side plank pose Superman pose Double legs lift Cool down
2	Sitting warm-up routine SS I (x4) Triangle pose Warrior II Reverse warrior pose Side angle pose Extended side angle pose Wide legged forward bend pose Tree pose Standing forward bend pose Table pose Cat & Cow pose Boat pose Rolling like a ball (x10) Double legs lift Dynamic bridge pose Cool down	Standing warm-up routine SS I (x4) Triangle pose Warrior II Reverse warrior pose Side angle pose Extended side angle pose Wide legged forward bend pose Tree pose Standing forward bend pose Table pose Cat & Cow pose Side plank pose Rolling like a ball (x10) Double legs lift Dynamic bridge pose Cool down
3	Sitting warm-up routine SS I (x5) Triangle pose Warrior I Warrior II Reverse warrior pose Side angle pose Extended side angle pose High lunge Revolved extended side angle pose Wide legged forward bend pose Tree pose Chair pose Side plank pose Dolphin pose Dolphin plank Rolling like a ball (x10) Double legs lift Dynamic bridge pose Cool down	Standing warm-up routine SS I (x5) Triangle pose Warrior I Warrior II Reverse warrior pose Side angle pose Extended side angle pose High lunge Revolved extended side angle pose Wide legged forward bend pose Tree pose Chair pose Side plank pose Dolphin pose Dolphin plank Rolling like a ball (x10) Double legs lift Dynamic bridge pose Cool down

Months	Mondays	Wednesdays
4	Sitting warm-up routine SS I (x6) Triangle pose Warrior II Reverse warrior pose Side angle pose Extended side angle pose High lunge pose Revolved side angle pose Wide legged forward bend pose Variation of tree pose Forward bending pose Seated forward bend pose Boat pose Side plank pose Sphinx pose Dolphin pose Dolphin plank Roll-up (x10) Crisscross crunches (x20) Dynamic bridge (x10) Cool down	Standing warm-up routine Cat & cow pose Extended puppy pose Spinal balance pose SS I (x6) Triangle pose Warrior II Reverse warrior pose Side angle pose Extended side angle pose High lunge pose Revolved side angle pose Wide legged forward bend pose Gate pose Low lunge pose Rolling like a ball (x5, Advanced x5) Plow pose Side-reclining leg lift pose Sphinx pose Dolphin pose Dolphin plank Bow pose Roll-up (x10) Crisscross crunches (x20) Dynamic bridge (x10) Cool down
5	Supine warm-up routine SS II (x4) Triangle pose Wide legged forward bend pose High lunge pose Variation of tree pose Chair pose Mermaid pose Side plank pose Side reclining leg lift pose Dolphin pose Advanced dolphin plank Bow pose Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x10) Cool down	Sitting warm-up routine SS II (x4) Triangle pose Wide legged forward bend pose High lunge pose Revolved side angle pose Standing forward bend pose Wide angle pose Rolling like a ball (x5) Plow pose Side plank pose Dolphin pose Advanced dolphin plank Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x10) Cool down

Months	Mondays	Wednesdays
6	Supine warm-up routine SS II (x5) Triangle pose Wide legged forward bend pose Revolved triangle pose Intense side stretch pose Eagle pose Standing forward bend pose Camel pose Side plank pose Bow pose Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x20) Cool down	Sitting & Standing warm-up routine SS II (x5) Triangle pose Wide legged forward bend pose Revolved side angle pose Revolved triangle pose Intense side stretch pose Eagle pose Wide angle pose Cow face Rolling like a ball (x5) Fish pose Plow pose Shoulder stand pose Advanced Dolphin plank Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x20) Cool down
7	Supine warm-up routine SS II (x6) Triangle pose Wide legged forward bend pose Revolved triangle pose Half moon pose Warrior III Tree pose Standing forward bend pose Camel pose Side plank pose Bow pose Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x20) A single leg bridge Cool down	Sitting & Standing warm-up routine SS II (x6) Triangle pose Wide legged forward bend pose Revolved side angle pose Revolved triangle pose Half moon pose Warrior III Eagle pose Wide angle pose Cow face pose Rolling like a ball (x5) Fish pose Plow pose Shoulder stand pose Roll-up (x20) Crisscross crunches (x40) Dynamic bridge (x20) A single leg bridge Cool down
8	Supine warm-up routine SS II (x7) Triangle pose Wide legged forward bend pose Revolved triangle pose Half moon pose Warrior III Eagle pose Standing forward bend pose Camel pose Side plank pose Dolphin pose Advanced Dolphin plank Bow pose Roll-up (x20) Crisscross crunches (x60) Dynamic bridge (x20) A single leg bridge Cool down	Standing warm-up routine SS II (x7) Triangle pose Wide legged forward bend pose Revolved side angle pose Revolved triangle pose Half moon pose Warrior III Eagle pose Gate pose Low lunge pose Side plank pose Rolling like a ball (x5) Fish pose Plow pose Shoulder stand pose Roll-up (x20) Crisscross crunches (x60) Dynamic bridge (x20) A single leg bridge & Cool down

Appendix G.

Yoga Preconsiderations

Pre-considerations for Yoga Exercise

1) Remember that you are responsible for your own body and personal safety.
2) Please tell me about any medical or other conditions (cold, flu & muscle soreness etc) that may affect your performance before class begins.
3) Do not wear tight clothes that restrict breathing, digestion, or circulation.
4) Minimize jewelry, buttons, zippers, belts, pockets, etc.
5) Do not hold your breath during the postures as this will cause strain.
6) Before you begin your Yoga postures, it is important to recognize your body's capabilities.
7) Women should avoid doing inversions such as shoulder stand, and plow postures when they are menstruating.
8) I recommend that you can eat a light meal such as fruit, energy bars, or yogurt from 30 to 60 minutes before class.

Class Policies:

1) Attendance is expected. If you have to miss Yoga sessions, please let me know.
2) Please try not to be late to class, it may distract your classmates, and also you may be easily injured without warm-up.
3) Makeup sessions may be offered as needed on Thursdays only.
4) No classes during holidays and university vacations and 64 Yoga sessions will be offered (See Yoga Session Schedule).
5) Record your Heart Rate and RPE every Wednesdays.

Other Considerations:

1) The recommendation calcium intake is 1000 mg/day. Since adequate calcium intake is critical for building bone density, it is very important that participants consuming less than 1000 mg/day increase calcium intake by consuming foods high in calcium content such as dairy products (milk or yogurt) or take a calcium supplement.
2) During the Yoga exercise study, please do not begin any new exercise programs outside of this class as this may affect your bone density.
3) Since dramatic weight loss is associated with bone loss, please do not begin any caloric restriction diets during this study

Appendix H.

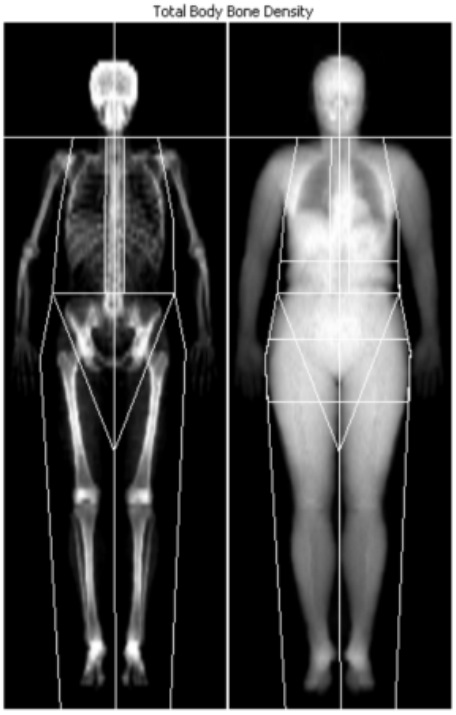
Sample DXA and pQCT scans

OU Bone Density Research Laboratory

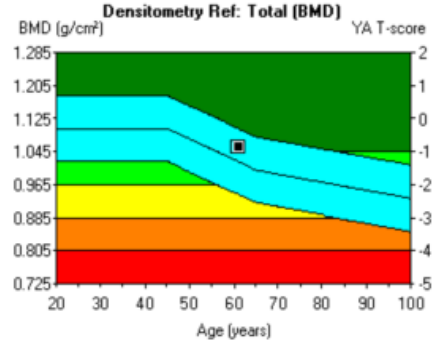
1401 Asp Ave
Norman, OK 73019

Patient ID:	**Facility ID:**			
Birth Date:	**Referring Physician:**			
Height / Weight:	**Measured:**	4/26/2010	4:01:23 PM	(13.31)
Sex / Ethnic:	**Analyzed:**	4/26/2010	5:06:36 PM	(13.31)

Total Body Bone Density



Densitometry Ref: Total (BMD)



Region	BMD [1] (g/cm²)	Young-Adult [2] (%)	Young-Adult [2] T-score	Age-Matched [3] (%)	Age-Matched [3] Z-score
Total	1.057	94	-0.9	104	0.5

COMMENTS:

Image not for diagnosis

Printed: 6/7/2010 3:14:13 PM (13.31)76:0.15:153.85:31.2 0.00:-1.00
4.80x13.00 12.2:%Fat=27.1%
0.00:0.00 0.00:0.00
Filename: 4u4i1lb93.dfb
Scan Mode: Standard 0.4 µGy

1 -Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for Total Body Total)
2 -USA (Combined NHANES (ages 20-30) / Lunar (ages 20-40)) Total Body Reference Population (v112)
3 -Matched for Age, Weight (females 25-100 kg), Ethnic

GE Healthcare

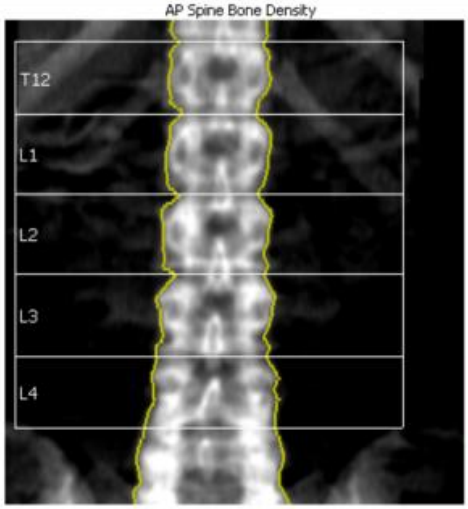
Lunar Prodigy
DF+14583

OU Bone Density Research Laboratory

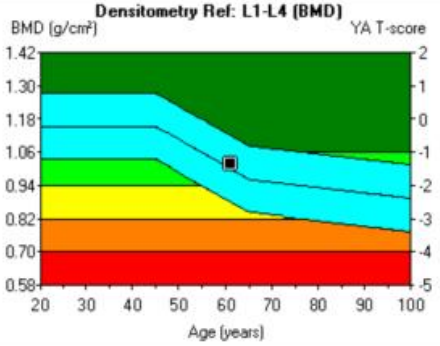
1401 Asp Ave
Norman, OK 73019

Patient ID:	**Facility ID:**			
Birth Date:	**Referring Physician:**			
Height / Weight:	**Measured:**	4/26/2010	4:03:45 PM	(13.31)
Sex / Ethnic:	**Analyzed:**	4/26/2010	5:05:00 PM	(13.31)

AP Spine Bone Density



Densitometry Ref: L1-L4 (BMD)



Region	BMD[1] (g/cm²)	Young-Adult[2] (%)	Young-Adult[2] T-score	Age-Matched[3] (%)	Age-Matched[3] Z-score
L1-L4	1.017	86	-1.4	102	0.1

COMMENTS:

Image not for diagnosis

Printed: 6/7/2010 3:30:54 PM (13.31)76:3.00:50.00:12.0 0.00:8.52 0.60:1.05
16.6:%Fat=13.4%
0.00:0.00 0.00:0.00
Filename: o35i1lb93.dfs
Scan Mode: Standard 37.0 µGy

1 - Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for AP Spine L1-L4)
2 - USA (Combined NHANES (ages 20-30) / Lunar (ages 20-40)) AP Spine Reference Population (v112)
3 - Matched for Age, Weight (females 25-100 kg), Ethnic
11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women: Normal = T-score at or above -1.0 SD; Osteopenia = T-score between -1.0 and -2.5 SD; Osteoporosis = T-score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-scores.)

GE Healthcare

Lunar Prodigy
DF+14583

OU Bone Density Research Laboratory

1401 Asp Ave
Norman, OK 73019

Patient ID:	**Facility ID:**			
Birth Date:	**Referring Physician:**			
Height / Weight:	**Measured:**	4/26/2010	4:08:10 PM	(13.31)
Sex / Ethnic:	**Analyzed:**	4/26/2010	4:08:17 PM	(13.31)

DualFemur Bone Density

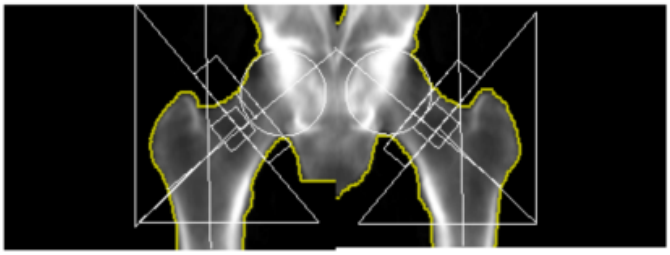
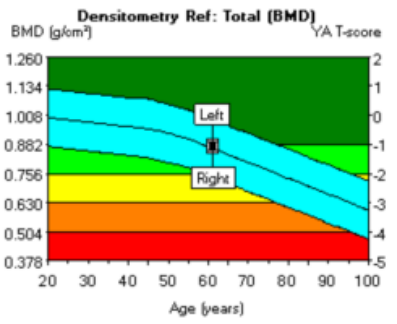


Image not for diagnosis



HAL chart results unavailable

Region	BMD [1] (g/cm²)	Young-Adult [2,7] (%)	Young-Adult [2,7] T-score	Age-Matched [3] (%)	Age-Matched [3] Z-score
Total					
Left	0.880	87	-1.0	102	0.1
Right	0.865	86	-1.1	100	0.0
Mean	0.873	87	-1.1	101	0.1
Difference	0.016	2	0.1	2	0.1

COMMENTS:

1 - Statistically 68% of repeat scans fall within 1SD (± 0.010 g/cm² for DualFemur Total)
2 - USA (Combined NHANES (ages 20-30) / Lunar (ages 20-40)) Femur Reference Population (v112)
3 - Matched for Age, Weight (females 25-100 kg), Ethnic
7 - DualFemur Total T-score difference is 0.1. Asymmetry is None.
11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women: Normal = T-score at or above -1.0 SD; Osteopenia = T-score between -1.0 and -2.5 SD; Osteoporosis = T-score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-scores.)

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GE Healthcare

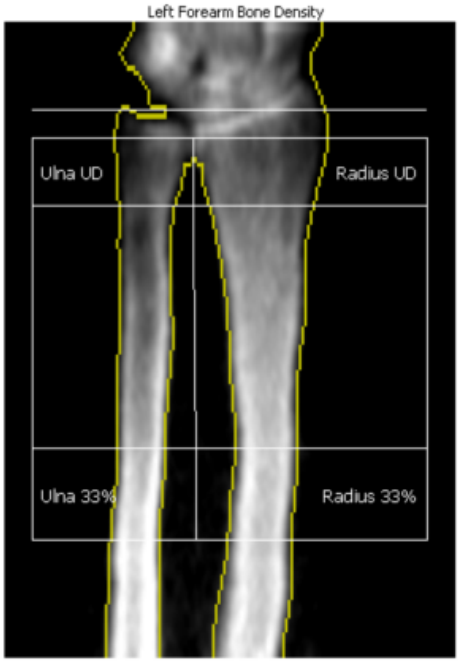
Lunar Prodigy
DF+14583

OU Bone Density Research Laboratory

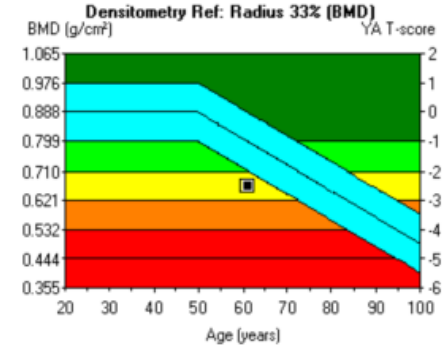
1401 Asp Ave
Norman, OK 73019

Patient ID:	**Facility ID:**			
Birth Date:	**Referring Physician:**			
Height / Weight:	**Measured:**	4/26/2010	4:11:22 PM	(13.31)
Sex / Ethnic:	**Analyzed:**	4/26/2010	4:11:26 PM	(13.31)

Left Forearm Bone Density



Densitometry Ref: Radius 33% (BMD)



Region	BMD [1] (g/cm²)	Young-Adult [2] (%)	Young-Adult [2] T-score	Age-Matched [3] (%)	Age-Matched [3] Z-score
Radius 33%	0.665	75	-2.5	83	-1.5

COMMENTS:

Image not for diagnosis

Printed: 6/7/2010 3:37:58 PM (13.31)76:0.15:50.00:12.0 0.00:6.20 0.60:1.05
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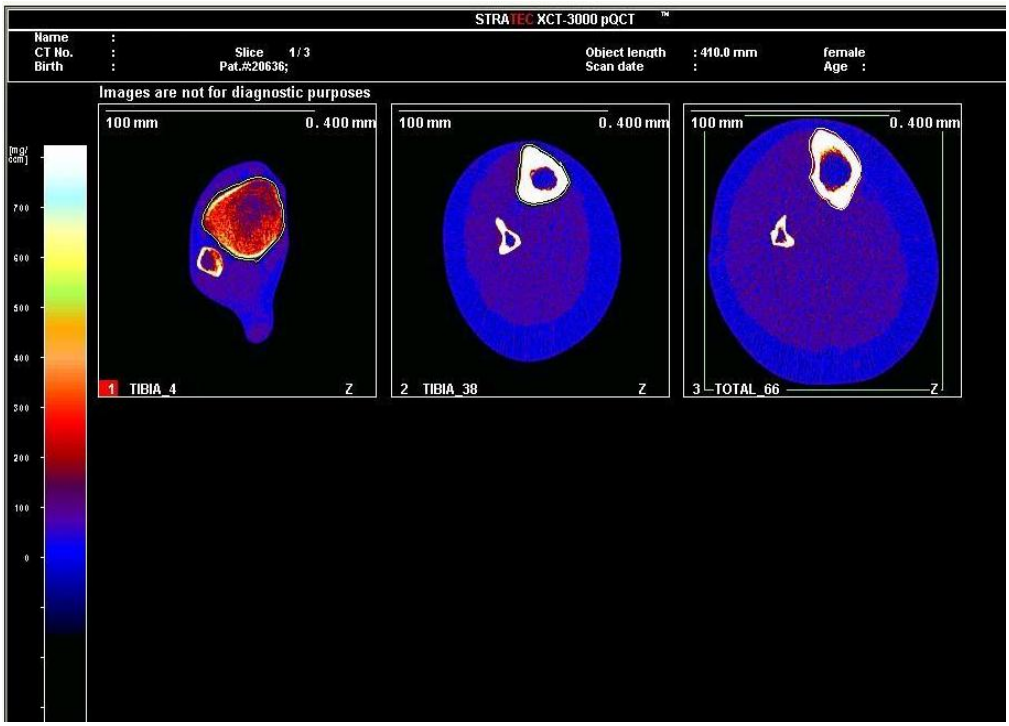
0.00:0.00 0.00:0.00
Forearm Length: 25.5 cm
Filename: zh5i1lb93.dfa
Scan Mode: Standard 2.0 µGy

1 - Statistically 68% of repeat scans fall within 1SD (± 0.020 g/cm² for Left Forearm Radius 33%)
2 - USA (Combined NHANES (ages 20-30) / Lunar (ages 20-40)) Forearm Reference Population (v11Z)
3 - Matched for Age, Ethnic
9 - Lunar calibration in use.
11 - World Health Organization - Definition of Osteoporosis and Osteopenia for Caucasian Women: Normal = T-score at or above -1.0 SD; Osteopenia = T-score between -1.0 and -2.5 SD; Osteoporosis = T-score at or below -2.5 SD; (WHO definitions only apply when a young healthy Caucasian Women reference database is used to determine T-scores.)

GE Healthcare

Lunar Prodigy
DF+14583

pQCT Tibia 4%, 38% 66%



Appendix I.

Bone ALP and TRAP5b Instructions

MicroVue™ BAP EIA Summary

Reagents and Samples Preparation

- ☐ Dilute 10X Wash Buffer 1:10 with DI Water

Assay Procedure

Pipette 125 µL of Assay Buffer into assay wells

↓

Pipette 20 µL of the Standards, Controls, and Specimens into assay wells *(Gently swirl plate to ensure mixing of sample and buffer.)*

↓

Incubate **3 hours ± 10 min** at 20 – 28°C

Wash **4 times** with 1X Wash Buffer

- ☐ Prepare Substrate Solution *(30 – 60 min before use)*
  Add one Substrate tablet per bottle of Substrate Buffer (Shake vigorously)

↓

Pipette 150 µL Substrate Solution

↓

Incubate **30 ± 5 min** at 20 – 28°C

↓

Pipette 100 µL Stop Solution

↓

Read the Optical Density at 405 nm.
Analyze the assay results using a quadratic curve fit
$y = A + Bx + Cx^2$

INTENDED USE

The MicroVue™ BAP immunoassay provides a quantitative measure of bone-specific alkaline phosphatase (BAP) activity in serum as an indicator of osteoblastic activity. Measurement of BAP is intended for use as an aid in the:

- management of postmenopausal osteoporosis and Paget's disease;
- monitoring of postmenopausal women on hormonal or bisphosphonate therapy;
- prediction of skeletal response to hormonal therapy in postmenopausal women.

SUMMARY AND EXPLANATION

The skeletal, or bone-specific, isoform of alkaline phosphatase is a tetrameric glycoprotein found on the cell surface of osteoblasts.[1] Osteoblasts are the cells responsible for synthesis of new bone matrix and its mineralization. The function of BAP has not been fully elucidated, though its role in skeletal mineralization has been confirmed.[1,2,3]

Bone is constantly undergoing a metabolic process called remodeling.[3,4] This includes a degradation process, bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoblasts.[3,4] Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is, resorption and formation are in balance.[3,4] In abnormal states of bone metabolism this process becomes uncoupled and, when resorption exceeds formation, this results in a net loss of bone which can lead to osteoporosis,[3,4] or to the disordered bone tissue of pagetic lesions.[5] The measurement of specific biochemical markers of these remodeling events provides analytical data regarding the rate of bone metabolism or "turnover."[3,4]

Osteoporosis is a metabolic bone disease characterized by abnormal bone remodeling. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in susceptibility to fractures.[6] The most common type of osteoporosis occurs in postmenopausal women as a result of the estrogen deficiency produced by the cessation of ovarian function.[3] Restoration of premenopausal estrogen levels by replacement therapy prevents bone loss and osteoporosis.[3,6] Estrogens and a class of compounds known as bisphosphonates are antiresorptive therapies which can be used to prevent bone loss or treat osteoporosis.[3,6,7]

Osteoporosis can also result from attaining an inadequate peak bone mass during the growing years, an age-related imbalance of bone remodeling with a net excess of resorption, and a number of clinical conditions and therapies which induce bone loss or bone remodeling imbalances.[3] These include endocrine diseases such as hypogonadism, hyperthyroidism, hyperparathyroidism, and hypercortisolism; renal failure; cancers metastatic to bone; gastrointestinal diseases related to nutrition and mineral metabolism; connective tissue diseases; multiple myeloma; chronic immobilization, alcoholism, or tobacco use; and chronic therapy with heparin or corticosteroids.[3]

Paget's disease of bone is a focal disorder resulting in pain and skeletal deformity in symptomatic patients.[5] Pagetic lesions are characterized by bone matrix of highly abnormal structure arising from excessive rates of remodeling activity. The lesions occur predominantly in the skull, spine, pelvis and long bones, and can result in fractures and neurological impairment.[5] The etiology of Paget's disease is unknown but hypotheses involving genetic and viral factors are compelling.[5] Bisphosphonates and calcitonin are currently used to suppress the high rate of biochemical activity to normal levels, enabling restoration of normal bone structure.[9]

As a quantitative measure of a marker of bone turnover, BAP provides useful information on bone remodeling in osteoporosis and Paget's disease, and changes in disease activity produced by antiresorptive therapy.[10-12] For the MicroVue BAP assay, antibody technology was employed to produce a monoclonal antibody that demonstrates specificity for BAP.[10] The specificity of the monoclonal antibody used in the assay allows for simple, convenient, reproducible and direct quantitation of BAP activity in serum.

PRINCIPLE OF THE PROCEDURE

MicroVue BAP is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a pNPP substrate.

REAGENTS AND MATERIALS PROVIDED

96 Assays for Bone-specific Alkaline Phosphatase

MicroVue BAP EIA contains the following:

A–F Standards: Parts 4395 – 4400 — 0.4 mL, 1 each
(A = 0, B = 2, C = 20, D = 50, E = 80, F = 140 U/L BAP)
BAP purified from osteosarcoma SAOS-2 cells in a buffered solution containing magnesium chloride, zinc sulfate, surfactant, carrier protein, blue dye, and sodium azide (0.05%) as a preservative

L, H Low/High Controls Parts 4401, 4402 — 0.4 mL, 1 each
BAP purified from osteosarcoma SAOS-2 cells in a buffered solution containing magnesium chloride, zinc sulfate, surfactant, carrier protein, blue dye, and sodium azide (0.05%) as a preservative

❶ **Coated Strips** Part 4660 — 12 each
Purified murine monoclonal Anti-BAP IgG antibody adsorbed onto stripwells

❷ **Stop Solution** Part 4702 — 15 mL
0.5N NaOH

❸ **10X Wash Buffer** Part 4703 — 55 mL
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative

❹ **Assay Buffer** Part 4403 — 27 mL
A buffered solution containing magnesium chloride, zinc sulfate, surfactant, and sodium azide (0.05%) as a preservative

❺ **Substrate Buffer** Part 4404 — 3 x 10 mL
A 2-amino-2-methyl-1-propanol solution containing HEDTA, magnesium chloride, zinc sulfate, and sodium azide (0.05%) as a preservative

❻ **Substrate Tablets** Part 0012 — 3 x 20 mg
p-Nitrophenyl phosphate

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 20 µL and 100–300 µL
- Items suitable for liquid measurement of 100–300 mL
- Container for wash buffer dilution
- Deionized or distilled water
- Plate reader capable of Optical Density readings at A_{405} > 2.0
- Quadratic calibration curve fitting software

WARNINGS AND PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7. Test each sample in duplicate.
8. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
9. 0.5N NaOH is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
10. Sodium azide is used as a preservative. Incidental contact with or ingestion of buffers containing sodium azide may cause irritation to the skin, eyes, or mouth. Only use buffers for intended purposes and avoid contact with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up.
11. The substrate buffer contains 2-amino-2-methyl-1-propanol and may cause irritation to the eyes and/or skin with prolonged contact. Contacted areas should be immediately washed with soap and water.
12. Use of multichannel pipets or repeat pipetors is recommended to ensure timely delivery of reagents.
13. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
14. Dilute samples greater than 140 U/L in Assay Buffer and retest. Include the dilution factor in the final calculation.
15. This assay may be performed with any validated washing method.

REAGENT PREPARATION

Bring reagents and materials for the assay to 20–28°C before use. After removing the needed reagents and materials, return unused items to 2–8°C.

Coated Strips

Remove Stripwell Frame and the required number of Coated Strips from the pouch (See table in *ASSAY PROCEDURE* Section). Ensure that the pouch containing any unused strips is completely resealed.

Wash Buffer

Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at 20–28°C. Use 1X Wash Buffer within 21 days of preparation.

Working Substrate Solution

Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix. Discard remaining Working Substrate Solution after use.

STORAGE

Store kit at 2–8°C. Do not freeze. Store unused reagents at 2–8°C. Equilibrate reagents to 20–28°C before use. Store 1X Wash Buffer (10X diluted) at 20–28°C.

SPECIMEN COLLECTION AND STORAGE

Collect serum using standard venipuncture technique. Specimens should be collected without anticoagulants and in such a way to avoid hemolysis. Allow the blood to clot and separate the serum by centrifugation. Serum can be stored for 5 days at 2–8°C, at ≤ -40°C for 12 months, or at ≤ -80°C for 36 months. Do not subject samples to more than 3 freeze/thaw cycles.

"Off the clot" serum, serum separator tube serum, Na heparin plasma, and Li heparin plasma yield substantially equivalent results. It is recommended that plasma samples not be prepared using chelating agents such as EDTA or citrate.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See REAGENT PREPARATION before proceeding.

Determine amount of each reagent required for the number of strips to be used.

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
Substrate (bottle)	1	1	2*	2*
1X Wash Buffer (mL)	100	150	200	300

*When more than one bottle or vial is to be used, combine the contents and mix prior to use.

Sample Incubation

1. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table) just prior to use. Ensure that the foil pouch containing any unused strips is completely resealed.
2. Place desired number of Coated Strips in the Stripwell Frame. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.
3. Add 125 µL Assay Buffer to each well.
4. Add 20 µL of Standards, Controls, and Specimens to assay wells. **Do not** mix with Assay Buffer by repeat pipetting. This step should be completed within 30 minutes. **Gently swirl plate to ensure mixing of sample and buffer.**
5. Incubate for 3 hours (± 10 minutes) at 20–28°C.
6. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C Substrate Buffer (see table). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

Washing Step

7. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at 20–28°C. Use 1X Wash Buffer within 21 days of preparation.
8. Manually invert/empty strips. Add at least 250 µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash.

Substrate Incubation

9. Add 150 µL of Working Substrate Solution to each well. Discard remaining Working Substrate Solution after use.
10. Incubate for 30 minutes (± 5 minutes) at 20–28°C.

Stop/Read

11. Add 100 µL of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
12. Read the optical density at 405 nm. Assure that no large bubbles are present in the wells and that the bottoms of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.
13. Quantitation software with a quadratic calibration curve fitting equation **must** be used to analyze the MicroVue BAP assay results.

    Equation: $y = A + Bx + Cx^2$

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel. The optical density values are provided and are to be used as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

If the optical density of the MicroVue BAP Standard F is less than 1.0, the results should be considered questionable and the samples should be repeated.

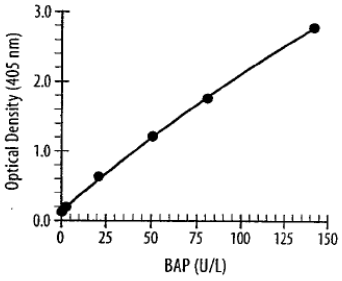
INTERPRETATION OF RESULTS

Sample results are expressed as U/L and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).

In the MicroVue BAP assay, 1 Unit represents 1 µmol of pNPP hydrolyzed per minute at 25°C in 2-amino-2-methyl-1-propanol buffer.

Representative Standard Curve

Standard BAP levels: 0, 2, 20, 50, 80, 140 U/L



LIMITATIONS OF THE PROCEDURE

HAMA Interference

Some individuals have antibodies to mouse antibodies (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice. In particular, it has been reported that serum samples from patients who have undergone therapy or diagnostic procedures that include infusion of mouse monoclonal antibody may produce erroneous results. Therefore, MicroVue BAP results for such patients should be used only in conjunction with results from some other diagnostic procedure and with information available from the clinical evaluation of the patient.

Samples with significant elevations of liver alkaline phosphatase activity may cause aberrantly elevated results in the MicroVue BAP assay.

Paget's patients who have low levels of disease may have bone-specific alkaline phosphatase levels that fall within the MicroVue BAP reference range.

SAMPLE VALUES

BAP reference ranges have been established for normal males over 25 years of age (n = 126), normal premenopausal females between the ages of 25 and 44 (n = 178), and normal postmenopausal females (n = 107). For the purposes of establishing reference ranges, normal subjects were defined as:

- Basically healthy, no bone, endocrine or chronic disorders
- Regular menstrual cycles (premenopausal females)
- Not pregnant or breast feeding (females)
- Not currently taking any medication known to influence bone metabolism

Values may be influenced by such factors as low estrogen production, low calcium intake or low physical activity.[8] Estrogen deficiency in postmenopausal women can result in elevated bone turnover.[3,4] Each laboratory should establish its own normal reference range. The ranges are expressed as nonparametric reference intervals (90% CI).

	Age (Yr)		Range (U/L)	Median
Females	25 - 44	Premenopausal	11.6 - 29.6	18.3
Females	≥ 45	Postmenopausal	14.2 - 42.7	25.0
Males	≥ 25		15.0 - 41.3	23.2

PERFORMANCE CHARACTERISTICS

Antibody Specifications

The bone-specific alkaline phosphatase antibody has selective, high affinity for the bone-specific alkaline phosphatase isoform, low cross-reactivity to the liver form of alkaline phosphatase, and negligible binding of intestinal and placental isoenzymes.

AP Isoenzyme	% Reactivity
Bone	100
Liver	3 – 8
Placental	0
Intestine	0.4

Sensitivity

The minimum detection limit of the MicroVue BAP assay is 0.7 U/L, determined by the upper 3 SD limit in a zero standard precision study.

Recovery - Spike Recovery

Spike recovery was determined by adding a known quantity of purified BAP to serum samples with different levels of endogenous BAP. Typical results are provided after spiking serum samples with low, medium, and high concentrations of BAP and assaying in triplicate.

Endogenous (U/L)	Added (U/L)	Observed (U/L)	Recovery (%)
13.4	15.7	29.1	99
17.6	37.5	55.3	99
27.2	57.2	88.1	106

Recovery - Linearity

Linearity was determined by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed (U/L)	Expected (U/L)	Recovery (%)
1	neat	108.5	-	-
	1:2	51.1	54.2	94
	1:4	25.8	27.1	99
	1:6	18.0	18.1	99
2	neat	39.1	-	-
	1:2	20.1	19.5	103
	1:4	10.3	9.8	105
	1:6	6.7	6.5	103
3	neat	58.4	-	-
	1:2	29.9	29.2	102
	1:4	15.7	14.6	108
	1:6	9.7	9.7	100

Precision

Within-run precision was determined for ≥ 21 replicates of 3 samples on 3 plates from each of 3 kit lots (9 plates total). Between-run precision was determined for 3 samples run in 6 separate plates from each of 3 kit lots (18 plates total). Typical results are provided below.

BAP (U/L BAP)	Within-run[1] CV%	Between-run[2] CV%
12	5.8	5.2
35	3.9	7.6
100	5.2	5.0

[1] n=21 [2] n=6 runs

Interfering Substances

The following substances were tested at the specified concentrations, and were found not to interfere with the assay:

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin	25 mg/dL
Triglycerides	1420 mg/dL
Total Protein	6.0 g/dL †
Total Protein	15.6 g/dL †
Total Protein	6.0 g/dL ‡
Total Protein	15.6 g/dL ‡

† Protein with water

‡ Protein with BAP (BAP concentration = 43.6 U/L)

Drug Interferences

Various concentrations of drugs were added to three separate serum pools containing approximately 35, 70, and 105 U/L BAP and assayed in triplicate. The following drugs with the highest concentrations tested were found not to interfere with the assay:

Substance	Highest Concentration
Etidronate	350 µg/mL
Estrogen	100 µg/mL
Ibuprofen	150 µg/mL
Acetominophen	350 µg/mL
Aspirin	350 µg/mL
Calcitonin - Human	80 µg/mL
Calcitonin - Salmon	80 µg/mL
Calcium	500 µg/mL
Norethindrone/ethynil estradiol mixture (oral contraceptive)	3 mg/mL
Vitamin D	400 IU/mL

Accuracy

Comparative studies were performed to assess the correlations between measurements of serum bone-specific alkaline phosphatase (BAP) obtained using the MicroVue BAP assay to results obtained using three currently marketed methods for measuring total alkaline phosphatase (TAP) or BAP. The studies were conducted at an independent clinical investigational site, utilizing sera from 114 patients with Paget's disease and 464 healthy subjects. The first comparative method was a colorimetric technique for the measurement of TAP. The correlation coefficient (r) obtained between this colorimetric method and the MicroVue BAP assay was 0.99. The second comparative method was an electrophoresis method for the determination of BAP isoenzyme levels (r = 0.99). The third comparative method was an immunoradiometric assay for the measurement of BAP (r = 0.99). Of the 114 patients diagnosed with Paget's disease, 101 patients had values greater than the upper limit of the reference ranges for the MicroVue BAP assay. Thirteen patients had values less than the upper limit of the reference ranges.

CLINICAL STUDIES

Use of MicroVue BAP for Monitoring the Efficacy of Antiresorptive Therapy in Osteoporosis

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the MicroVue BAP assay to monitor changes in serum BAP concentrations associated with amino-bisphosphonate (alendronate) antiresorptive therapy. Subjects, drawn from a larger study of the efficacy of alendronate for treating osteoporosis,[7] were postmenopausal women, aged 45 to 84 years (mean 64 ± 7 years), diagnosed with osteoporosis (based on clinical presentation or baseline lumbar spine bone mineral density [LSBMD] more than 2.5 standard deviations below the mean for mature premenopausal women). At baseline, eligible subjects were randomized to receive either 10 mg alendronate and 500 mg calcium per day (ALN) or placebo and 500 mg calcium per day (CTL). Serum specimens were obtained at baseline, 3, 6 and 12 months from all subjects.

MICROVUE Bone Health

TRAP5b

ENZYME IMMUNOASSAY KIT

For Research Use Only.
Not for use in diagnostic procedures.

Catalog No. 8033

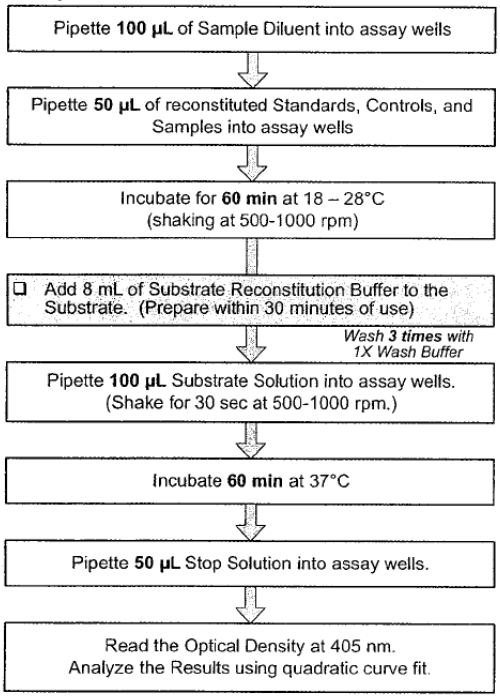
MicroVue™ TRAP5b Enzyme Immunoassay Summary

Standards and Controls Preparation

- ❑ Reconstitute Standards with 300 µL of D.I. water. (Prepare Standards within 2 hours)
- ❑ Reconstitute Controls with 300 µL of D.I. water. (Prepare Controls within 2 hours)
- ❑ Dilute 10X Wash Buffer 1:10 with D.I. water.

NOTE: Mix Standards gently with pipette; do not vortex.

Assay Procedure



PURPOSE OF THE TEST

The MicroVue TRAP5b Assay is an immunocapture enzyme ass for determination of tartrate-resistant acid phosphatase isoform 5 (TRAcP 5b). TRAP5b is derived from osteoclasts in human serum.[5] This kit is intended for research use only.

Features

- The total assay time is two hours.
- The kit measures only active TRAP5b enzyme activity.
- Samples do not require pre-dilution.

SUMMARY AND EXPLANATION

TRAP5b (serum band 5 tartrate-resistant acid phosphatase, TRA 5b; EC 3.1.3.2) is a 35-37 kDa glycoprotein. TRAP5b is typica expressed in proportion to osteoclast activity and is secreted in the circulation. Research indicates that serum TRAP5b is a potentia useful serological marker for bone resorption.[9]

The MicroVue TRAP5b Assay Kit detects the enzyme activity TRAP5b based on an immuno-captured enzyme assay system.[9]

Elevated serum TRAP5b levels are thought to be associated wi active bone remodelling. Increased serum levels are observe during normal bone growth among healthy children. Elevat serum TRAP5b levels have also been detected in certain disea states and conditions characterized by increased bone resorption.[2] Examples are: Paget's disease of bone, hemodialysis, prima hyperparathyroidism, metastatic malignancies involving bo resorption, multiple myeloma, and bilaterally ovariectomiz women. Post-menopausal women on estrogen replacement thera typically have lower levels in serum than untreated postmenopau women; therefore, specific determination of TRAP5b activity may a potential means for the measurement and monitoring of chang in bone metabolism in response to therapy.

PRINCIPLE OF THE PROCEDURE

The MicroVue TRAP5b Assay is a 2-step, direct capture, 96-well E Serum or plasma samples and reconstituted Standards and Contr are added to coated microwell plate wells along with Samp Diluent.[1-3]

Naturally occurring, inactive TRAP5b fragments in the serum m interfere with the detection of TRAP5b in physiological sampl The MicroVue TRAP5b Assay avoids the influence of the inacti fragments by using two different monoclonal antibodies. T assay employs two unique monoclonal antibodies, Trk49 and Trk generated with immunization of purified TRAP5b from human bo cells. The first antibody, Trk49, is highly specific to inactive TRAP fragments; the second antibody, Trk62, is highly specific for inta active TRAP5b. Trk49 binds inactive TRAP5b fragments, there making Trk62 more available to bind active TRAP5b in the microw The resulting TRAP5b assay is one that is specific and has go precision and wide range of linearity.

After the immunoreaction incubation, the plate is washed to remove unbound material, and a prepared substrate, 2-chloro-4-nitrophenyl phosphate (CNPP, pH 6.4), is added to the wells. Since the TRAP5b analyte is itself an enzyme, a labeled secondary antibody-enzyme conjugate is not required. At the end of this incubation, the reaction is stopped with the addition of a 0.2N NaOH solution and read via microplate reader at 405 nm. The TRAP5b activity is then calculated off a quadratic curve. The amount of color developed is proportional to the concentration of TRAP5b in the samples.

REAGENTS AND MATERIALS PROVIDED

40 Assays for TRAP5b conducted in duplicate (96 wells)

MicroVue TRAP5b Assay kit contains the following:

A B C D E **TRAP5b Standards:** **Items 0711631-71** **2 x 0.3 mL each**
(lyophilized) Human TRAP5b. The exact concentration is stated on each vial

L H **Controls** **Items 0711681-91** **2 x 0.3 mL each**
(lyophilized) Human TRAP5b. The concentration range is stated on the kit Certificate of Analysis (C of A)

❶ **Microwell Plate** **Item 0711611** **12 each**
12 x 8 wells coated with murine monoclonal anti-TRAP5b antibodies

❷ **Stop Solution** **Item 07116C1** **8 mL**
0.2N sodium hydroxide (NaOH)

❸ **10X Wash Buffer** **Item 07116D1** **40 mL**
TBS/Tween. Contains 0.5% Tween® 20 and 0.02% ProClin® 300

❹ **Sample Diluent** **Item 0711621** **15 mL**
Tris buffer. Contains 0.02% ProClin 300

❺ **Substrate Reconstitution Buffer** **Item 07116B1** **20 mL**
MES buffer. Contains 0.02% ProClin 300

❻ **Substrate** **Item 07116A1** **2 x 8 mL**
Substrate dissolving solution, 2-chloro-4-nitrophenyl-phosphate powder (CNPP)

Plate Tape Cover **Item 0047** **3 each**

Tween® 20 is a registered trademark of ICI Americas Inc.
ProClin® is a registered trademark of Rohm and Haas Company.

MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable micropipettes for dispensing 50, 100, 300 µL, both single and multi-channel
- Microplate shaker capable of constant shaking at 500 – 1000 rpm for 60 minutes
- Incubator at 37°C
- Labware suitable for liquid measurement of 10-300 mL
- Deionized or distilled water
- Microplate reader capable of reading at 405 nm
- Computer with CD ROM Drive
- Software package facilitating data generation, quadratic curve fit, and data analysis
- Suitable device for washing the microplate
- Graduated pipette or equivalent for dispensing 8 mL
- Absorbent material for blotting the in-process microplate after washing

WARNINGS AND PRECAUTIONS

1. Research Use Only. Not for Use in Diagnostic Procedures.
2. This kit contains components of human origin. These materials were found to be non-reactive for HIV, HCV, and HBsAg. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
3. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
4. Dispose of containers and unused contents in accordance with National and Local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. Do not use Coated Strips if pouch is punctured.
8 Test each sample in duplicate.
9. Wear gloves and eye protection when handling contents of this kit. Use good laboratory practices to reduce exposure.
10. 0.2N NaOH acts as an irritant and can cause irritation to exposed areas. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
11. Avoid contact with the irritant Substrate Solution, which contains CNPP. In case of accidental contact, immediately wash skin thoroughly with soap and water.
12. ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Seek medical attention if symptoms are experienced.
13. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
14. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
15. Perform this assay with any validated washing method. Do not wash wells with a multi-channel pipette
16. Generate a standard curve with each assay.
17. Standard concentrations are assigned for each lot. Read label on each Standard vial or Certificate of Analysis carefully for specific concentrations.

REAGENT PREPARATION

All reagents should be equilibrated to 18-28°C prior to use. Prepare assay reagents as follows:

Sample Diluent

Sample Diluent is provided ready to use.

Standards

Add 300 µL of deionized (distilled) water to the vial containing lyophilized Standard and dissolve for at least 5 minutes. Mix thoroughly. The reconstituted Standards should be used within 2 hours if stored at 18-28°C or within 24 hours if stored at 4°C.

Controls

Add 300 µL of deionized (distilled) water to the vials containing lyophilized Controls, and dissolve for at least 5 minutes. Mix thoroughly. The reconstituted Controls should be used within 2 hours if stored at 18-28°C or within 24 hours if stored at 4°C.

10X Wash Buffer

Dilute 40 mL of 10X Wash Buffer with 360 mL deionized (distilled) water. The working Wash Buffer is stable for 1 month at 18-28°C.

Substrate Solution

Prepare Working Substrate Solution by adding 8 mL of Substrate Reconstitution Buffer. Prepare within 30 minutes of use.

Stop Solution

Stop Solution is provided ready to use.

STORAGE

Store the kit at 2-8°C. Store unused reagents at 2-8°C. Under these conditions, assay components are stable until the expiry date printed on the kit label.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (Heparin) can be used as samples in the MicroVue TRAP5b Assay. Collect serum using standard venipuncture technique, avoiding hemolysis. Allow the blood to clot, and separate the serum by centrifugation.

Samples can be stored up to 8 hours at room temperature, up to 2 days at 2-8°C, one month at -20°C, and at -80°C for long-term storage. Do not subject samples to more than 3 freeze/thaw cycles.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

Sample/Enzyme Incubation

1. Allow pouch of Coated Strips to equilibrate to 18-28°C before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch. Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.
2. Pipette 100 µL of Sample Diluent into microplate wells.
3. Pipette 50 µL of each reconstituted Standard, Control and sample into appropriate microplate wells.
4. Seal the microwell plate with supplied plate tape cover and incubate for 60 minutes at 18-28°C on a microplate shaker set at 500 – 1000 rpm. 1060 ↳21
5. After incubation, wash the microplate wells three times with a minimum of 300 µL of Wash Buffer per well. After washing, tap the wells gently on a paper towel to expel any remaining liquid.

Substrate Incubation

6. Pipette 100 µL of Working Substrate Solution into each well.
7. Seal the microplate and mix on a microplate shaker for 30 seconds at 500 – 1000 rpm. After shaking, incubate for 60 minutes in a 37°C incubator.

Stop/Read

8. Pipette 50 µL of Stop Solution into each well to stop the reaction.
9. Read and record the absorbance of each well at 405 nm.
10. Use a quadratic curve fit for the standard curve. Calculate the values of Controls and specimens from the standard curve.

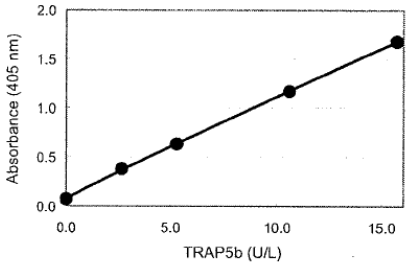
QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values provided are intended as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be tested again.

INTERPRETATION OF RESULTS

Representative Standard Curve



OBSERVED VALUES

Observed serum values for TRAP5b activity in healthy men and women are reported as follows:

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

Observed TRAP5b values (U/L) in 64 healthy adults (see gender and age information below) using both serum and plasma (Heparin) collection methods. Plasma samples were run for comparison to serum results.

- 28 Men, ages 25-54 (mean: 35.4)
- 36 Women, ages 21-59 (mean: 41.9)

Sample Type	Mean (U/L)	Min	Max	Correlation (r)
Serum	3.5 ± 1.4	1.2	6.7	–
Heparin Plasma	3.6 ± 1.4	1.2	7.3	0.989

PERFORMANCE OF THE TEST

Typical analytical data of MicroVue TRAP5b Assay are presented in this section. For kit lot-specific standard curve and controls values see the Certificate of Analysis.

Sensitivity

The minimum detection limit of the MicroVue TRAP5b assay is 0.2 U/L, determined by the upper 3 SD limit in a zero standard precision study.

Precision

- Intra assay (Within Run) (n = 16)

Sample	Mean (U/L)	Standard Deviation (U/L)	%CV
1	3.4	0.07	2.2
2	7.4	0.14	1.9

- Inter assay (Run to Run) (n = 8)

Sample	Mean (U/L)	Standard Deviation (U/L)	%CV
1	3.8	0.11	3.0
2	7.4	0.15	2.0

Spike Recovery

Spike recovery of 92-103% was determined by adding a known quantity of purified TRAP5b to serum samples with different levels of endogenous TRAP5b.

Linearity

Linearity was performed by serially diluting serums with sample diluent and comparing observed values with expected values.

Sample	Dilution Factor	Observed (U/L)	Expected (U/L)	Recovery (%)
1	neat	3.7	-	-
	1:2	1.8	1.8	95.9
	1:4	0.9	0.9	95.1
	1:8	0.5	0.5	101.2
2	neat	7.7	-	-
	1:2	3.8	3.8	99.8
	1:4	1.9	1.9	97.5
	1:8	0.9	1.0	97.4
3	neat	12.0	-	-
	1:2	5.8	6.0	96.2
	1:4	3.0	3.0	100.8
	1:8	1.4	1.5	95.9

Interfering Substances

The following substances were tested at the specified concentrations and were found not to interfere with the assay:

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin F	20 mg/dL
Bilirubin C	20 mg/dL
Lipids (Intralipid®)	2500 Turbidity
RF (Rheumatoid Factor)	500 U/mL

Intralipid® is a registered trademark of Fresenius Kabi AB.

CUSTOMER ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

Additional information about Quidel and Quidel's products and distributors can be found on our website at www.quidel.com.

Appendix J.

IGF-I Instruction

Intended Use

For In Vitro Diagnostic Use

The IDS IGF-I ELISA kit is a two-site immunoenzymometric assay [IEMA] for the quantitative determination of Insulin-like Growth Factor I in human serum or plasma. The method incorporates a sample pre-treatment to avoid interference from binding proteins.

Summary and Explanation

Insulin-like growth factor I (IGF-I) is a polypeptide of 70 amino acids (7650 daltons), and is one of a number of related insulin-like growth factors present in the circulation. The molecule shows approximately 50% sequence homology with proinsulin and has a number of biological activities similar to insulin. The peptide is growth hormone (GH) dependent to a high degree, but there is growing evidence of GH-independent secretion. IGF-I has numerous growth-promoting effects, including mitogenic effects and the promotion of cartilage sulphation. It also mediates growth promoting actions of growth hormone on skeletal and other body fluids.

Almost all (>95%) of serum IGF-I circulates bound to specific IGF binding proteins, of which six classes (IGF-BPs 1-6) are now recognised. BP3 is thought to be the major binding protein of IGF-I, forming a ternary complex of 140 000 daltons with IGF-I and an acid-labile sub-unit.

The measurement of serum IGF-I is of recognised value in children with growth disorders and in the diagnosis and monitoring of acromegaly. IGF-I concentrations change with age, nutritional status, body composition and growth hormone secretion.

A single basal IGF-I determination is useful in the assessment of short stature in children and in nutritional support studies of acutely ill patients. For the diagnosis of acromegaly, a single IGF-I determination is considered more reliable than a random GH determination.

Method Description

Patient samples are incubated briefly with a reagent to inactivate binding proteins, and then diluted for assay. In the IDS IGF-I ELISA kit a purified sheep polyclonal anti-IGF-I is coated onto the inner surface of polystyrene microtitre wells (the solid phase or capture antibody). The pre-treated, diluted sample is then incubated, together with horseradish peroxidase-labelled monoclonal anti-IGF-I, in antibody coated wells for 2 hours at room temperature. The wells are washed and a single component chromogenic substrate (a formulation of tetramethyl-benzidine) is added to develop colour. The absorbance of the stopped reaction mixture is read in a microtitre plate reader, colour intensity developed being directly proportional to the amount of IGF-I present in the sample.

Warnings and Precautions

The IDS IGF-I ELISA kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IDS Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Human Serum: Calibrators [CAL] and Controls [CTRL] Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled in accordance at Biosafety Level 2.

Sodium Azide

Xn. Harmful: Calibrators [CAL] and Controls [CTRL] contain sodium azide (NaN_3) >0.1% (w/w) (<1%).

R22 Harmful if swallowed.

R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S46 If swallowed, seek medical advice immediately and show this container or label.

S36/37 Wear suitable protective clothing and gloves.

S60 This material and/or its container must be disposed of as hazardous waste.

Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Tetramethylbenzidine

TMB Substrate [SUBS] contains 3,3',5,5'-tetramethylbenzidine.

R21/22 Harmful by contact with skin and if swallowed.

S36/37 Wear suitable protective clothing and gloves.

1M sulphuric acid

Stop Solution [H_2SO_4] contains 1M sulphuric acid.

R36/38 Irritating to eyes and skin.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37 Wear suitable protective clothing and gloves.

Preparation of Reagents

Calibrators [CAL] and Controls [CTRL]: Calibrators [CAL] and Controls [CTRL] are supplied lyophilised. Reconstitute with 0.5 mL of distilled or deionised water, replace stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution.

If Calibrators [CAL] or Controls [CTRL] are to be used more than once, they must be frozen (-20°C) promptly (<30 minutes post-reconstitution).

When re-using frozen Calibrators [CAL] and Controls [CTRL], thaw at room temperature, with mixing, and use within 15 minutes of completion of thaw.

Wash Solution: Add the contents of each bottle of Wash Concentrate [WASHBUF] [20x] to 950 mL of distilled or de-ionised water and mix. Store at room temperature.

All other reagents are supplied ready for use.

Allow all reagents to come to room temperature before use. Reagents should be mixed by repeated inversion prior to use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Reconstituted Calibrators [CAL] and Controls [CTRL] can be stored at -20°C for up to 8 weeks.

Indications of possible deterioration of kit reagents

1. The presence of abnormal particulate matter in any of the reagents.
2. A decrease in the maximum absorbance.
3. A shift in the slope of the curve from its normal position.

Specimen Collection and Storage

The assay should be performed using either serum or plasma specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C. Avoid repeated freeze/thaw of samples

Note: Improper handling and storage of samples may result in loss of assayable IGF-I.

Procedure

Materials Provided

1. **[CAL] 0 - 5 - Calibrators ([REF] AC-2701A - AC-2701F):**
   Lyophilised human serum containing IGF-I and <1% sodium azide (0.09% reconstituted). The exact value of each Calibrator is printed on the bottle label, 0.5 mL per bottle, 6 bottles per kit.

2. **[MICROPLAT] - Antibody Coated Plate ([REF] AC-2702W):**
   Microplate with sheep anti-IGF-I polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant.

3. **[ENZYMCONJ] - Enzyme Conjugate ([REF] AC-2703):**
   Phosphate buffered saline containing mouse anti-IGF-I monoclonal antibody linked to horseradish peroxidase, protein, enzyme stabilisers and preservative. 22 mL per bottle.

4. **[CTRL] 1 - 2 – Controls ([REF] AC-2705A - AC-2705B):**
   Lyophilised human serum containing IGF-I and <1% sodium azide (0.09% reconstituted), 0.5 mL per bottle, 2 bottles per kit.

5. **[H_2SO_4] - Stop Solution ([REF] AC-2706):**
   1M sulphuric acid, 12 mL per bottle.

6. **[RELEASREAG] - Releasing Reagent ([REF] AC-2707):**
   Proprietary reagent for dissociating IGF-I from binding proteins, 21 mL per bottle.

7. **[SUBS] - TMB Substrate ([REF] AC-SUBS):**
   A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 30 mL per bottle.

8. **[SAMPDIL] - Sample Diluent ([REF] AC-270B):**
   Phosphate-buffered saline containing preservative, 50 mL per bottle.

9. **[WASHBUF] [20x] - Wash Concentrate ([REF] AC-WASHL):**
   Phosphate buffered saline containing Tween, 50 mL per bottle.

Materials Required but not Provided

1. Disposable 12 x 75 mm plastic tubes.
2. Precision pipetting devices to deliver 25 µL and 50 µL.
3. Repeating pipettes to deliver 100 µL and 1 mL, e.g. Eppendorf Multipipette 4780, or similar.
4. Precision multi-channel pipettes to deliver 100 µL and 200 µL.
5. Vortex mixer.
6. Automatic microplate washer (optional).
7. Photometric microplate reader and data analysis equipment.

Assay Procedure

Reconstitute or prepare reagents as described in "Preparation of Reagents".

1. Prepare labelled plastic tubes, one for each Calibrator [CAL], Control [CTRL] and sample.
2. Add **25 µL** of each Calibrator [CAL], Control [CTRL] or sample to appropriately labelled tubes.
3. Add **100 µL** of Releasing Reagent [RELEASREAG] to each tube. Vortex all tubes. Incubate at 18-25°C for 10 minutes.
4. Add **1.0 mL** of Sample Diluent [SAMPDIL] to each tube. Vortex all tubes. Samples are now ready to assay.
5. Add **50 µL** of each diluted Calibrator, Control or sample to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.

   Note: these should be dispensed within a period of 10 minutes to minimise drift.
6. Add **200 µL** of Enzyme Conjugate [ENZYMCONJ] to all wells using a multichannel pipette. Incubate at 18-25°C for between 2 hours and 2 hours 15 minutes.
7. Wash all wells three times with Wash Solution:
   a. Automatic plate wash: Set plate washer to dispense at least **300 µL** of Wash Solution per well. Fill and aspirate for 3 cycles.
   b. Manual wash: Decant the contents of the wells by inverting sharply. Dispense **250 µL** of Wash Solution to all wells. Decant and repeat twice.

   Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution before proceeding to the next step.
8. Add **200 µL** of TMB Substrate [SUBS] to all wells using a multichannel pipette. Incubate at 18-25°C for 30 minutes.

   Note: TMB Substrate is easily contaminated. Only remove the required amount for the assay from the bottle. Dispose of unused TMB Substrate. Do not return to bottle.
9. Add **100 µL** of Stop Solution [H_2SO_4] to all wells using a multichannel pipette.
10. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution.

Calibration

The IDS IGF-I ELISA kit has been calibrated against the International Reference Reagent for Insulin-like Growth Factor-I, IGF-I, coded 87/518.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results

Calculate the mean absorbance for each Calibrator, Control and unknown sample. Prepare a calibration curve on log-log graph paper by plotting the mean absorbance for each Calibrator on the ordinate against concentration of IGF-I on the abscissa. Read values for each control and unknown sample from the calibration curve in µg/L.

For the estimation of low values we recommend a plot of absorbance against concentration of low calibrator values on linear-linear graph paper. Results for unknown samples can be read directly from the curve.

Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. Smoothed spline curve fits are recommended.

Conversion of units:

$$X\ \mu g/L \quad \overset{\times\ 0.131}{\Rightarrow} \quad Y\ nmol/L$$

$$X\ \mu g/L \quad \overset{\times\ 7.63}{\Leftarrow} \quad Y\ nmol/L$$

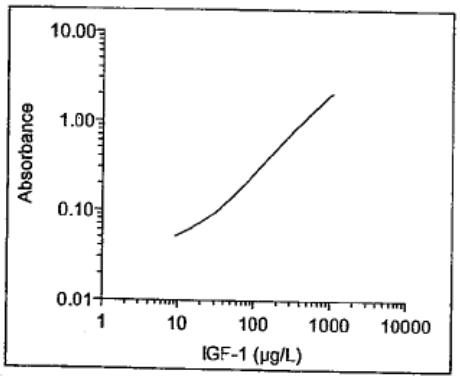
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result.

Well	Description	Duplicate Absorbance	Mean Absorbance	Result (µg/L)
A1, A2	Calibrator 0 (0 µg/L)	0.060 0.060	0.060	
B1, B2	Calibrator 1 (16 µg/L)	0.108 0.114	0.111	
C1, C2	Calibrator 2 (32 µg/L)	0.152 0.152	0.152	
D1, D2	Calibrator 3 (107 µg/L)	0.347 0.357	0.352	
E1, E2	Calibrator 4 (357 µg/L)	0.923 0.933	0.928	
F1, F2	Calibrator 5 (1137 µg/L)	2.231 2.238	2.235	
G1, G2	Sample 1	0.253 0.263	0.258	71
H1, H2	Sample 2	0.664 0.698	0.681	244

Typical Calibration Curve

This sample calibration curve is for illustration only.



Limitations of Use

1. Samples suspected of containing analyte concentrations in excess of the highest calibrator should be assayed in dilution.
2. As in the case of any diagnostic procedure results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
3. The following substances have been tested and found not to interfere in the IDS IGF-I assay:

Haemoglobin	tested up to 500 mg/dL
Bilirubin	tested up to 30 mg/dL
Lipid	tested up to 4000 mg/dL triglyceride

4. The hook effect was tested using concentrations of IGF-I up to 5,000 µg/L. No hook effect was observed.

Expected Values

The following ranges have been determined using the IDS IGF-I ELISA kit and are provided for guidance only. Each laboratory should determine ranges for their local population.

	Age (years)	n	µg/L
Normal range	<1	10	12 - 108
	1 - 3	14	13 - 100
	>3 - 6	19	26 - 280
	>6 - 9	16	85 - 230
	>9 - 12	25	98 - 404
	>12 - 15	17	142 - 525
	>15 - 20	14	146 - 415
	>20 - 30	18	89 - 276
	>30 - 40	18	22 - 197
	>40 - 50	18	49 - 147
	>50 - 60	26	35 - 210
	>60 - 70	25	30 - 196
	>70	11	56 - 191
Acromegalics		10	340 - 635

Note: The expected values presented for each sample type are absolute ranges - each range is from the minimum value to the maximum value for each respective sample type.

Performance Data

Accuracy

The IDS IGF-I ELISA kit was compared against a recognised two-site sandwich immunoradiometric assay for the quantitative determination of IGF-I. A population of 67 samples, selected to represent a wide range of IGF-I [12-627 µg/L], were assayed by each method. Least squares regression analysis was performed on the comparative data;

$y = 0.45 [x] - 11.82$; correlation coefficient (r) = 0.94.

Precision

Intra-Assay Variation (n=20)		Inter-Assay Variation (n=38)	
Mean Value (µg/L)	%CV	Mean Value (µg/L)	%CV
22.9	7.2	26.7	6.5
132	5.1	145	5.7
273	4.6	287	4.3

Sensitivity

The sensitivity, defined as the concentration corresponding to the mean plus 2 standard deviations of 20 replicates of the zero Calibrator, is 3.1 µg/L.

Linearity

Linearity was assessed by diluting a high patient sample with a low patient sample.

Sample	Measured (µg/L)	Expected (µg/L)	% M/E
A	61.0	-	-
0.875A+0.125B	82.1	83.8	98
0.750A+0.250B	106	107	100
0.625A+0.375B	129	129	100
0.500A+0.500B	145	152	95
0.375A+0.625B	174	175	100
0.250A+0.750B	203	198	103
0.125A+0.875B	225	221	102
B	244	-	-
		Mean	**99**

Inactivation of IGF-I Binding Proteins

Thirty five samples with IGF-I concentrations from 10.5-512 µg/L were assayed comparing acid-ethanol extraction and IDS Releasing Reagent. Acid ethanol results were 92.9% of Releasing Reagent results (CV 20.5%).

Recovery

Recovery was assessed by adding IGF-I to samples prior to assay.

Sample conc. (µg/L)	IGF-I added (µg/L)	Measured (µg/L)	Recovery (µg/L)	Recovery (%)
221	300	526	305	102
231	300	506	275	92
187	300	456	269	90
			Mean	**95**

Specificity

Potential cross-reactivity and interference by IGF-II, insulin and proinsulin were tested by adding up to 4,444 µg/L IGF-II, 25 IU/L insulin and 1000 µg/L proinsulin to "0" and "150" µg/L Calibrators. No interference was observed.

Appendix K.

Calcium recommendation

Calcium Recommendation

Bone Density Laboratory
Health and Exercise Science Department

Subject ID: _____-

Recommended Calcium intake: ____1000_mg/day Your Calcium intake: _____mg/day

♦ The calcium content of foods, including fresh fruits and vegetables, can be found on the USDA National Nutrient Database Web site at http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl

♦ Calcium Containing Foods By Calcium Content Per Serving with 100 mg (USDA, 2002)

Food	Serving size	Calcium, mg
>400 mg		
Tofu, regular with calcium sulfate	½ cup	434
Tofu, firm with calcium sulfate	½ cup	860
Fortified cereal	¾ cup	varies by brand
300–400 mg		
Whole milk	1 cup	291
Milkshake	8 oz.	300
Lowfat yogurt	8 oz.	300
Fortified orange juice	1 cup	300
Fortified soy milk	1 cup	300
Fortified rice milk	1 cup	300
Skim, 1%, or 2% milk	1 cup	321
Fortified cereal	¾ cup	varies by brand
Fortified oatmeal	1 pkt	350
200–300 mg		
Cheddar, monterey or provolone cheese	1 oz.	206
Soybeans, roasted	1 cup	237
Spinach (cooked)	1 cup	245
Mixed cheese dish	1 cup	250
Fortified energy bar	1	250
Soybeans (cooked)	1 cup	261
Swiss cheese	1 oz.	272
Plain yogurt	8 oz	274
100–200 mg		
Pizza	1 slice	100
Fortified waffles	2	100
Fortified butter or margarine	1 Tbsp.	100
Sherbet	1 cup	103
Mustard greens (cooked), Bok Choy	1 cup	104
Spaghetti, lasagna	1 cup	125
Cottage cheese	1 cup	138
Baked beans	1 cup	142
Dandelion greens or turnip greens (cooked)	1 cup	147
Ice cream	1 cup	151
Frozen yogurt or pudding	½ cup	152
American, Feta, or Mozzarella cheese	1 oz.	174
Soybeans, boiled	1 cup	175

Appendix L.

Data

ID	GROUP	AGE	HEIGHT	preWT	postWT	pcWT	preFAT	postFAT	pcFAT	prePCFAT	postPCFAT
1	1	48.80	159.50	72.10	68.50	-4.99	27.85	23.96	-13.97	39.00	35.10
7	1	49.30	168.00	75.00	83.50	11.33	30.45	38.34	25.91	40.80	46.10
8	1	47.50	169.50	77.80	79.50	2.19	32.19	32.43	.75	41.50	41.00
9	1	42.40	165.00	61.50	58.50	-4.88	22.85	19.24	-15.80	37.60	33.20
10	1	41.70	163.50	61.80	60.00	-2.91	25.52	23.36	-8.46	41.80	39.10
11	1	45.40	158.60	99.30	99.30	.00	45.98	47.34	2.96	46.70	48.10
17	1	42.40	164.50	74.70	79.40	6.29	32.50	37.41	15.11	44.00	47.70
18	1	41.40	161.50	90.30	91.50	1.33	42.73	45.03	5.38	48.00	49.40
19	1	47.10	161.00	68.10	70.30	3.23	28.87	30.65	6.17	42.00	43.70
20	1	37.80	152.00	51.00	51.50	.98	18.66	17.05	-8.63	37.10	33.90
22	1	50.90	165.50	72.40	71.00	-1.93	28.07	25.93	-7.62	39.10	36.80
23	1	50.10	160.00	52.80	53.50	1.33	12.47	12.50	.24	23.60	23.40
25	1	49.70	168.50	79.90	81.50	2.00	33.86	34.65	2.33	42.80	42.80
26	1	45.20	162.00	63.00	67.20	6.67	25.20	27.12	7.62	40.30	40.60
28	1	43.80	155.50	54.90	59.00	7.47	21.63	24.91	15.16	40.00	42.30
29	1	48.10	169.50	60.80	61.00	.33	16.16	15.94	-1.36	26.60	25.90
1	2	42.10	163.00	66.30	63.00	-4.98	22.08	18.96	-14.13	34.00	30.20
2	2	50.10	166.00	67.10	68.20	1.64	22.00	22.72	3.27	33.00	33.30
3	2	47.20	166.00	68.80	68.50	-.44	28.03	27.81	-.78	40.50	41.00
4	2	47.80	161.50	54.60	62.90	15.20	15.31	21.50	40.43	28.10	34.20
6	2	38.60	154.50	81.30	81.50	.25	38.77	37.63	-2.94	47.80	46.90
7	2	45.70	162.00	73.30	71.00	-3.14	34.62	31.51	-8.98	47.20	44.80
8	2	37.20	158.00	58.60	66.50	13.48	22.03	28.90	31.18	38.10	43.90
9	2	44.50	164.00	79.70	79.60	-.13	36.04	36.66	1.72	46.10	46.80
11	2	47.20	167.50	71.90	68.00	-5.42	24.55	21.74	-11.45	34.30	31.80
12	2	41.70	165.00	57.10	58.50	2.45	14.84	16.04	8.09	26.10	27.40
13	2	43.60	156.50	56.50	57.00	.88	17.94	18.81	4.85	31.80	33.00
14	2	37.80	154.10	77.90	73.40	-5.78	37.35	32.67	-12.53	48.10	45.00
15	2	44.00	163.00	79.00	78.50	-.63	30.08	28.66	-4.72	38.30	36.70
16	2	43.80	150.00	75.90	78.60	3.56	40.00	40.76	1.90	53.50	52.40
17	2	46.00	157.50	78.70	74.80	-4.96	37.28	31.96	-14.27	48.00	43.10
18	2	36.10	155.50	61.50	57.50	-6.50	18.42	16.01	-13.08	30.10	28.00
19	2	38.10	172.60	81.40	81.30	-.12	36.51	34.62	-5.18	45.80	43.10
20	2	46.40	157.50	70.90	73.70	3.95	31.85	32.50	2.04	45.50	44.60

ID	PCFATcov	pcPCFAT	preLEAN	postLEAN	pcLEAN	preCAL	postCAL	preBMI	postBMI	preTBMD	postTBMD
1	39.00	-10.00	40.64	41.45	1.99	586.00	707.00	28.30	26.90	1.25	1.3
7	40.80	12.99	41.70	42.09	.94	586.00	2242.00	26.60	29.60	1.16	1.2
8	41.50	-1.20	42.81	44.07	2.94	804.00	1014.00	27.10	27.70	1.15	1.2
9	37.60	-11.70	35.58	36.50	2.59	1329.00	1244.00	22.60	21.50	1.05	1.1
10	41.80	-6.46	33.49	34.38	2.66	1029.00	2253.00	23.10	22.40	1.03	1.0
11	46.70	3.00	49.51	48.13	-2.79	1133.00	1083.00	39.50	39.50	1.32	1.3
17	44.00	8.41	38.50	38.12	-.99	1225.00	1414.00	27.60	29.30	1.19	1.2
18	48.00	2.92	43.31	42.93	-.88	948.00	1743.00	34.60	35.10	1.33	1.3
19	42.00	4.05	37.37	37.06	-.83	893.00	1437.00	26.30	27.10	1.18	1.2
20	37.10	-8.63	29.48	31.21	5.87	541.00	1377.00	22.10	22.30	1.12	1.1
22	39.10	-5.88	41.04	41.98	2.29	566.00	800.00	26.40	25.90	1.17	1.2
23	23.60	-.85	38.30	38.87	1.49	1296.00	1443.00	20.60	20.90	1.07	1.1
25	42.80	.00	42.40	43.52	2.64	621.00	751.00	28.10	28.70	1.19	1.2
26	40.30	.74	34.84	37.28	7.00	1383.00	2661.00	24.00	25.60	1.13	1.2
28	40.00	5.75	29.92	31.47	5.18	705.00	975.00	22.70	24.40	1.21	1.2
29	26.60	-2.63	41.88	43.01	2.70	2759.00	1860.00	21.20	21.20	1.16	1.2
1	34.00	-11.18	40.30	41.24	2.33	247.00	549.00	25.00	23.70	1.20	1.2
2	33.00	.91	41.66	42.37	1.70	1110.00	1114.00	24.40	24.70	1.28	1.3
3	40.50	1.23	38.74	37.54	-3.10	817.00	666.00	25.00	24.90	1.09	1.1
4	28.10	21.71	36.68	38.80	5.78	666.00	1548.00	20.90	24.10	1.22	1.2
6	47.80	-1.88	40.23	40.72	1.22	1774.00	2093.00	34.10	34.10	1.10	1.1
7	47.20	-5.08	35.55	35.83	.79	587.00	1255.00	27.90	27.10	1.25	1.3
8	38.10	15.22	33.47	34.48	3.02	3244.00	2771.00	23.50	26.60	1.19	1.2
9	46.10	1.52	39.18	38.66	-1.33	1536.00	1620.00	29.60	29.60	1.27	1.3
11	34.30	-7.29	44.51	44.22	-.65	776.00	2364.00	25.60	24.20	1.16	1.1
12	26.10	4.98	39.50	39.91	1.04	467.00	455.00	21.00	21.50	1.17	1.2
13	31.80	3.77	36.18	35.91	-.75	1068.00	1422.00	23.10	23.30	1.09	1.1
14	48.10	-6.44	37.74	37.44	-.79	1102.00	2014.00	32.80	30.90	1.21	1.2
15	38.30	-4.18	45.46	46.44	2.16	2446.00	4146.00	29.70	29.50	1.27	1.3
16	53.50	-2.06	31.78	34.25	7.77	1481.00	1231.00	33.70	34.90	1.24	1.3
17	48.00	-10.21	37.69	39.66	5.23	881.00	1847.00	31.70	30.20	1.15	1.2
18	30.10	-6.98	40.43	38.93	-3.71	1200.00	883.00	25.40	23.80	1.17	1.2
19	45.80	-5.90	40.10	42.77	6.66	376.00	880.00	27.30	27.30	1.15	1.2
20	45.50	-1.98	35.64	38.06	6.79	1131.00	1827.00	28.60	29.70	1.11	1.1

ID	preRTHIPd	stRTHIPd	RTHIPcov	pcRTHIPd	preRFNd	postRFNd	pcRFNd	preRTCd	postRTCd	pcRTCd	preLTHd
1	1.15	1.15	1.15	.00	1.09	1.09	.00	.92	.92	.00	1.13
7	.96	.97	.96	1.04	.92	.91	-1.09	.78	.82	5.13	1.02
8	.86	.86	.86	.00	.89	.87	-2.25	.64	.64	.00	.92
9	.92	.91	.92	-1.09	.96	.93	-3.12	.74	.73	-1.35	.91
10	.80	.80	.80	.00	.80	.79	-1.25	.61	.60	-1.64	.77
11	1.23	1.21	1.23	-1.63	1.11	1.06	-4.50	1.04	1.02	-1.92	1.21
17	.96	.98	.96	2.08	.92	.93	1.09	.81	.82	1.23	.97
18	1.19	1.20	1.19	.84	1.12	1.16	3.57	1.03	1.02	-.97	1.21
19	.97	.98	.97	1.03	1.00	.99	-1.00	.73	.74	1.37	1.00
20	.88	.88	.88	.00	.86	.83	-3.49	.73	.71	-2.74	.91
22	1.12	1.11	1.12	-.89	1.08	1.08	.00	.89	.87	-2.25	1.05
23	.82	.81	.82	-1.22	.73	.72	-1.37	.64	.62	-3.13	.85
25	1.13	1.12	1.13	-.88	1.06	1.04	-1.89	.88	.88	.00	1.11
26	1.01	1.02	1.01	.99	1.03	1.03	.00	.82	.83	1.22	1.04
28	.94	.95	.94	1.06	.95	.97	2.11	.70	.70	.00	.98
29	.87	.88	.87	1.15	.90	.89	-1.11	.68	.70	2.94	.91
1	.95	.94	.95	-1.05	.88	.89	1.14	.73	.72	-1.37	.99
2	1.09	1.09	1.09	.00	1.00	1.01	1.00	.89	.89	.00	1.14
3	.99	.98	.99	-1.01	1.02	.98	-3.92	.75	.75	.00	.99
4	1.10	1.11	1.10	.91	1.09	1.09	.00	.89	.90	1.12	1.08
6	.97	.97	.97	.00	.96	.99	3.13	.75	.74	-1.33	.91
7	1.22	1.21	1.22	-.82	1.22	1.19	-2.46	.97	.97	.00	1.16
8	1.02	1.03	1.02	.98	1.09	1.03	-5.50	.81	.83	2.47	.99
9	1.01	1.03	1.01	1.98	1.04	1.07	2.88	.78	.81	3.85	1.09
11	.96	.93	.96	-3.12	.99	.99	.00	.62	.60	-3.23	.98
12	.98	.99	.98	1.02	.89	.89	.00	.71	.75	5.63	.99
13	.97	.98	.97	1.03	1.00	1.00	.00	.75	.76	1.33	.98
14	1.08	1.09	1.08	.93	1.06	1.10	3.77	.82	.85	3.66	1.11
15	1.17	1.18	1.17	.85	1.14	1.09	-4.39	.87	.90	3.45	1.10
16	1.14	1.15	1.14	.88	1.07	1.06	-.93	.91	.92	1.10	1.13
17	.93	.93	.93	.00	.83	.89	7.23	.70	.70	.00	.94
18	1.09	1.09	1.09	.00	1.03	1.00	-2.91	.84	.84	.00	1.05
19	1.03	1.05	1.03	1.94	.98	1.00	2.04	.83	.87	4.82	.99
20	.98	.96	.98	-2.04	.91	.92	1.10	.75	.73	-2.67	.93

ID	pcLTHd	preLFNd	postLFNd	pcLFNd	preLTCd	postLTCd	pcLTCd	preL14d	postL14d	pcL14d	preL24d
1	.00	1.08	1.06	-1.85	.91	.91	.00	1.37	1.33	-2.92	1.40
7	.98	1.00	.99	-1.00	.80	.82	2.50	1.06	1.06	.00	1.09
8	.00	.94	.96	2.13	.74	.73	-1.35	1.20	1.19	-.83	1.23
9	-1.10	.96	.93	-3.12	.73	.72	-1.37	1.07	1.08	.93	1.09
10	-2.60	.76	.75	-1.32	.57	.56	-1.75	.97	.99	2.06	.97
11	-2.48	1.10	1.07	-2.73	1.00	.96	-4.00	1.39	1.41	1.44	1.44
17	1.03	.93	.91	-2.15	.80	.80	.00	1.26	1.25	-.79	1.27
18	2.48	1.13	1.18	4.42	1.06	1.07	.94	1.50	1.50	.00	1.50
19	1.00	.98	.97	-1.02	.78	.77	-1.28	1.13	1.18	4.42	1.14
20	-1.10	.83	.83	.00	.73	.71	-2.74	1.18	1.17	-.85	1.20
22	.95	1.01	1.03	1.98	.85	.83	-2.35	1.21	1.16	-4.13	1.24
23	-2.35	.74	.73	-1.35	.64	.63	-1.56	1.02	1.01	-.98	1.04
25	.00	1.14	1.11	-2.63	.85	.85	.00	1.23	1.24	.81	1.26
26	.00	1.02	1.05	2.94	.84	.85	1.19	1.26	1.23	-2.38	1.29
28	-1.02	1.01	.96	-4.95	.71	.70	-1.41	1.39	1.46	5.04	1.40
29	-2.20	.92	.87	-5.43	.69	.69	.00	1.12	1.10	-1.79	1.15
1	-1.01	.90	.89	-1.11	.79	.77	-2.53	1.21	1.21	.00	1.25
2	.00	1.05	1.04	-.95	.94	.95	1.06	1.42	1.39	-2.11	1.47
3	.00	1.01	1.00	-.99	.77	.78	1.30	1.22	1.23	.82	1.22
4	.00	1.06	1.06	.00	.87	.87	.00	1.19	1.20	.84	1.21
6	.00	.91	.92	1.10	.70	.70	.00	1.18	1.21	2.54	1.22
7	.86	1.11	1.12	.90	.90	.90	.00	1.42	1.40	-1.41	1.44
8	.00	1.05	1.04	-.95	.79	.80	1.27	1.25	1.29	3.20	1.27
9	-.92	1.08	1.08	.00	.87	.84	-3.45	1.32	1.33	.76	1.35
11	.00	.99	1.00	1.01	.67	.69	2.99	1.05	1.03	-1.90	1.06
12	.00	.92	.93	1.09	.78	.78	.00	1.13	1.14	.88	1.15
13	-1.02	.98	.96	-2.04	.77	.77	.00	1.16	1.13	-2.59	1.18
14	.00	1.08	1.10	1.85	.84	.86	2.38	1.21	1.21	.00	1.25
15	2.73	1.00	1.07	7.00	.84	.86	2.38	1.36	1.32	-2.94	1.39
16	-1.77	1.07	1.07	.00	.91	.92	1.10	1.38	1.41	2.17	1.42
17	-3.19	.91	.91	.00	.72	.69	-4.17	1.20	1.18	-1.67	1.23
18	1.90	.97	.95	-2.06	.82	.85	3.66	1.23	1.23	.00	1.25
19	2.02	.91	.93	2.20	.81	.83	2.47	1.23	1.20	-2.44	1.25
20	.00	.93	.92	-1.08	.71	.69	-2.82	1.03	1.05	1.94	1.08

ID	pcL24d	preR33d	postR33d	reR33cov	pcR33d	preTBMC	postTBMC	pcTBMC	preRTHc	postRTHc	pcRTHc
1	-1.43	.85	.84	.85	-1.18	2911.00	2862.00	-1.68	32.65	32.90	.77
7	1.83	.83	.85	.83	2.41	2439.00	2719.00	11.48	30.38	31.43	3.46
8	-.81	.87	.89	.87	2.30	2574.00	2550.00	-.93	26.02	26.09	.27
9	.92	.77	.74	.77	-3.90	2275.00	2222.00	-2.33	27.19	26.84	-1.29
10	2.06	.90	.90	.90	.00	2068.00	2061.00	-.34	20.76	20.76	.00
11	1.39	.91	.90	.91	-1.10	2950.00	2996.00	1.56	39.50	38.82	-1.72
17	-.79	.88	.89	.88	1.14	2891.00	2961.00	2.42	29.76	30.44	2.28
18	2.00	.94	.90	.94	-4.26	3046.00	3147.00	3.32	36.68	37.08	1.09
19	3.51	.91	.89	.91	-2.20	2418.00	2415.00	-.12	28.45	28.46	.04
20	-.83	.82	.80	.82	-2.44	2118.00	2024.00	-4.44	23.16	22.78	-1.64
22	-5.65	.95	.93	.95	-2.11	2601.00	2638.00	1.42	35.86	35.15	-1.98
23	.00	.88	.88	.88	.00	2151.00	2147.00	-.19	25.82	25.70	-.46
25	.00	.97	.89	.97	-8.25	2765.00	2796.00	1.12	34.58	34.87	.84
26	-3.10	.92	.93	.92	1.09	2511.00	2460.00	-2.03	30.12	30.41	.96
28	5.71	.95	.90	.95	-5.26	2471.00	2507.00	1.46	26.57	27.21	2.41
29	-2.61	.82	.82	.82	.00	2632.00	2666.00	1.29	29.32	30.15	2.83
1	-1.60	.73	.74	.73	1.37	2509.00	2560.00	2.03	29.23	28.91	-1.09
2	-3.40	.89	.87	.89	-2.25	2995.00	3040.00	1.50	34.93	35.29	1.03
3	.82	.83	.86	.83	3.61	2359.00	2517.00	6.70	28.19	28.51	1.14
4	.00	.91	.91	.91	.00	2491.00	2490.00	-.04	30.77	30.97	.65
6	1.64	.78	.82	.78	5.13	2022.00	1959.00	-3.12	28.75	28.24	-1.77
7	-2.78	.93	.95	.93	2.15	3107.00	2959.00	-4.76	35.79	35.79	.00
8	3.15	.76	.79	.76	3.95	2356.00	2477.00	5.14	28.18	29.04	3.05
9	-.74	.88	.87	.88	-1.14	3034.00	3019.00	-.49	28.21	29.66	5.14
11	.00	.84	.84	.84	.00	2447.00	2505.00	2.37	31.57	30.92	-2.06
12	.00	.88	.88	.88	.00	2454.00	2492.00	1.55	29.33	29.84	1.74
13	-2.54	.85	.85	.85	.00	2233.00	2294.00	2.73	25.92	26.27	1.35
14	.00	1.04	1.04	1.04	.00	2551.00	2526.00	-.98	28.32	29.19	3.07
15	-5.04	.90	.90	.90	.00	3008.00	2969.00	-1.30	36.16	36.33	.47
16	.00	.89	.86	.89	-3.37	2935.00	2830.00	-3.58	31.35	32.23	2.81
17	-2.44	.86	.83	.86	-3.49	2705.00	2462.00	-8.98	27.55	27.50	-.18
18	.80	.85	.88	.85	3.53	2308.00	2290.00	-.78	28.95	28.95	.00
19	-.80	.78	.82	.78	5.13	3159.00	2904.00	-8.07	31.19	32.17	3.14
20	1.85	.92	.93	.92	1.09	2473.00	2363.00	-4.45	28.19	27.77	-1.49

ID	postRFNc	pcRFNc	preRTCc	postRTCc	pcRTCc	preLTHc	postLTHc	reLTHcov	pcLTHc	preLFNc	postLFNc
1	4.88	1.46	9.29	9.38	.97	32.50	32.61	32.50	.34	5.09	4.88
7	4.08	-2.86	9.57	10.83	13.17	32.15	32.63	32.15	1.49	4.44	4.51
8	4.11	-.96	6.65	6.63	-.30	27.87	27.52	27.87	-1.26	4.24	4.34
9	4.19	-.95	8.31	7.99	-3.85	26.82	26.10	26.82	-2.68	4.37	4.30
10	3.34	2.14	5.01	4.75	-5.19	20.34	20.22	20.34	-.59	3.16	3.10
11	5.25	-3.14	13.34	13.47	.97	38.85	37.65	38.85	-3.09	5.30	5.06
17	4.49	3.70	9.10	9.06	-.44	30.47	29.95	30.47	-1.71	4.57	4.45
18	5.14	5.33	12.85	12.74	-.86	38.49	39.60	38.49	2.88	5.08	5.33
19	4.36	-1.13	8.07	8.23	1.98	27.82	28.03	27.82	.75	4.37	4.33
20	3.46	-1.14	6.12	5.82	-4.90	23.58	23.63	23.58	.21	3.51	3.54
22	4.92	.41	12.45	11.61	-6.75	34.33	33.83	34.33	-1.46	4.53	4.60
23	3.53	.00	7.94	8.00	.76	25.89	25.85	25.89	-.15	3.48	3.43
25	4.47	.22	10.88	10.99	1.01	35.12	35.28	35.12	.46	4.95	4.92
26	4.59	1.32	8.87	9.26	4.40	30.51	30.87	30.51	1.18	4.59	4.67
28	4.22	.96	6.84	7.22	5.56	27.55	27.14	27.55	-1.49	4.47	4.35
29	4.49	.67	8.86	9.50	7.22	31.16	30.62	31.16	-1.73	4.52	4.27
1	4.49	.67	7.97	7.88	-1.13	30.54	30.25	30.54	-.95	4.52	4.38
2	4.87	2.74	11.58	11.85	2.33	37.43	37.76	37.43	.88	5.22	5.22
3	4.32	-1.59	7.23	7.70	6.50	28.92	29.74	28.92	2.84	4.44	4.41
4	4.70	-1.05	9.37	9.62	2.67	30.16	30.11	30.16	-.17	4.73	4.79
6	4.40	1.15	8.68	8.21	-5.41	26.91	26.97	26.91	.22	4.06	4.05
7	5.84	-1.52	9.52	9.80	2.94	34.15	34.55	34.15	1.17	5.77	5.63
8	4.70	.00	7.78	8.30	6.68	27.07	27.75	27.07	2.51	4.65	4.75
9	4.64	3.34	7.27	8.29	14.03	31.83	31.68	31.83	-.47	4.70	4.78
11	4.37	-.23	7.93	7.64	-3.66	31.08	31.48	31.08	1.29	4.53	4.57
12	4.80	.63	7.15	7.95	11.19	29.69	29.85	29.69	.54	4.84	4.96
13	4.17	2.46	7.15	7.35	2.80	27.42	27.40	27.42	-.07	4.18	4.06
14	4.73	-2.27	6.81	7.59	11.45	30.11	30.42	30.11	1.03	5.01	4.93
15	5.22	-4.40	10.75	11.13	3.53	32.83	33.84	32.83	3.08	4.85	4.99
16	4.71	3.06	8.56	9.08	6.07	31.54	31.49	31.54	-.16	4.57	4.66
17	4.29	2.39	7.10	7.23	1.83	28.47	28.04	28.47	-1.51	4.12	4.29
18	4.35	1.40	8.00	7.96	-.50	28.20	28.55	28.20	1.24	4.22	4.13
19	4.73	1.94	9.83	10.87	10.58	30.90	31.93	30.90	3.33	4.46	4.51
20	4.15	1.47	8.17	7.96	-2.57	26.71	26.75	26.71	.15	4.17	4.13

ID	preLTCc	postLTCc	reLTCcov	pcLTCc	preL14c	postL14c	pcL14c	preL24c	postL24c	pcL24c	preR33c
1	9.23	9.28	9.23	.54	80.25	78.78	-1.83	64.57	63.98	-.91	2.25
7	10.03	10.78	10.03	7.48	61.02	59.22	-2.95	49.47	48.34	-2.28	1.95
8	7.86	7.29	7.86	-7.25	66.82	64.99	-2.74	53.80	51.88	-3.57	2.07
9	8.09	7.30	8.09	-9.77	60.45	59.91	-.89	48.74	48.29	-.92	1.59
10	4.64	4.53	4.64	-2.37	49.92	50.02	.20	39.50	39.29	-.53	2.11
11	13.17	12.30	13.17	-6.61	74.25	75.22	1.31	59.10	60.00	1.52	2.29
17	8.76	8.56	8.76	-2.28	67.05	65.31	-2.60	53.54	52.10	-2.69	2.00
18	13.77	13.85	13.77	.58	83.34	84.97	1.96	64.71	67.44	4.22	2.15
19	7.68	7.25	7.68	-5.60	55.58	57.90	4.17	43.82	45.28	3.33	1.99
20	6.18	6.23	6.18	.81	52.37	51.78	-1.13	41.71	41.23	-1.15	1.80
22	12.32	11.27	12.32	-8.52	68.31	65.37	-4.30	55.39	52.44	-5.33	2.37
23	7.54	7.89	7.54	4.64	54.22	54.75	.98	44.43	44.53	.23	1.98
25	11.46	11.26	11.46	-1.75	69.56	71.48	2.76	54.84	55.72	1.60	2.18
26	9.45	9.83	9.45	4.02	66.66	66.95	.44	54.17	53.09	-1.99	2.11
28	7.25	6.82	7.25	-5.93	73.87	78.84	6.73	58.62	63.27	7.93	2.10
29	10.08	10.05	10.08	-.30	63.98	64.12	.22	51.88	51.92	.08	2.23
1	8.55	8.65	8.55	1.17	63.38	63.88	.79	50.17	50.44	.54	2.03
2	13.02	13.27	13.02	1.92	82.60	83.98	1.67	66.08	67.13	1.59	2.35
3	8.06	8.58	8.06	6.45	66.66	67.30	.96	52.48	53.30	1.56	1.87
4	8.92	8.97	8.92	.56	59.19	60.75	2.64	47.58	48.80	2.56	2.17
6	7.54	7.84	7.54	3.98	54.90	57.47	4.68	44.86	46.16	2.90	1.44
7	8.11	8.39	8.11	3.45	69.70	69.39	-.44	55.90	54.29	-2.88	2.53
8	7.29	7.82	7.29	7.27	61.85	63.61	2.85	49.57	51.11	3.11	1.87
9	8.95	8.66	8.95	-3.24	70.67	70.20	-.67	57.57	56.23	-2.33	2.08
11	8.84	9.55	8.84	8.03	52.81	52.21	-1.14	41.82	42.38	1.34	2.03
12	8.25	8.21	8.25	-.48	56.14	58.11	3.51	44.48	46.76	5.13	2.13
13	7.98	8.15	7.98	2.13	59.81	65.75	9.93	46.35	52.72	13.74	1.95
14	7.86	8.34	7.86	6.11	54.43	55.56	2.08	44.04	44.72	1.54	2.36
15	9.83	10.19	9.83	3.66	77.18	74.97	-2.86	61.57	59.59	-3.22	2.35
16	8.89	9.41	8.89	5.85	66.87	67.63	1.14	54.00	53.97	-.06	1.85
17	8.61	8.54	8.61	-.81	56.73	56.89	.28	45.33	45.58	.55	2.29
18	8.07	8.27	8.07	2.48	58.26	57.72	-.93	46.35	45.98	-.80	1.96
19	10.16	10.74	10.16	5.71	70.13	67.67	-3.51	54.85	54.32	-.97	1.90
20	7.35	7.23	7.35	-1.63	50.04	53.52	6.95	41.58	44.24	6.40	2.00

ID	pcR33c	preLPD	postLPD	pcLPD	preSP	postSP	preSPcov	pcSP	preBC	postBC	pcBC
1	4.00	43.58	38.14	-12.48	40.86	32.69	40.86	-20.00	19.07	21.79	14.26
7	1.54	35.41	38.14	7.71	27.24	35.41	27.24	29.99	19.07	19.07	.00
8	3.38	27.24	27.24	.00	19.07	35.41	19.07	85.68	13.62	16.34	19.97
9	-2.52	24.52	21.79	-11.13	27.24	27.24	27.24	.00	13.62	10.90	-19.97
10	-3.32	32.69	27.24	-16.67	24.52	24.52	24.52	.00	10.90	10.90	.00
11	2.62	46.31	38.14	-17.64	54.48	49.03	54.48	-10.00	19.07	16.34	-14.32
17	2.00	40.86	43.58	6.66	29.96	40.86	29.96	36.38	19.07	19.07	.00
18	1.40	43.58	43.58	.00	46.31	.00	46.31	-100.00	21.79	24.52	12.53
19	5.53	38.14	43.58	14.26	35.41	27.24	35.41	-23.07	19.07	21.79	14.26
20	.00	29.96	27.24	-9.08	29.96	27.24	29.96	-9.08	10.90	10.90	.00
22	-1.69	43.58	35.41	-18.75	35.41	29.96	35.41	-15.39	24.52	21.79	-11.13
23	-1.52	24.52	19.07	-22.23	21.79	21.79	21.79	.00	19.07	13.62	-28.58
25	3.21	40.86	43.58	6.66	40.86	40.86	40.86	.00	19.07	21.79	14.26
26	2.37	38.14	38.14	.00	27.24	32.69	27.24	20.01	16.34	19.07	16.71
28	1.90	24.52	32.69	33.32	16.34	27.24	16.34	66.71	5.45	5.45	.00
29	-2.69	21.79	21.79	.00	21.79	32.69	21.79	50.02	10.90	10.90	.00
1	-.99	40.86	35.41	-13.34	40.86	32.69	40.86	-20.00	13.62	13.62	.00
2	-4.26	38.14	43.58	14.26	35.41	38.14	35.41	7.71	19.07	21.79	14.26
3	2.14	38.14	32.69	-14.29	35.41	38.14	35.41	7.71	13.62	13.62	.00
4	1.84	40.86	40.86	.00	32.69	32.69	32.69	.00	21.79	21.79	.00
6	5.56	40.86	40.86	.00	40.86	43.58	40.86	6.66	13.62	10.90	-19.97
7	-3.16	49.03	38.14	-22.21	40.86	32.69	40.86	-20.00	13.62	13.62	.00
8	-2.67	43.58	43.58	.00	29.96	27.24	29.96	-9.08	21.79	21.79	.00
9	-.48	40.86	38.14	-6.66	40.86	38.14	40.86	-6.66	19.07	21.79	14.26
11	.49	21.79	21.79	.00	24.52	27.24	24.52	11.09	10.90	10.90	.00
12	-.94	38.14	32.69	-14.29	35.41	40.86	35.41	15.39	16.34	13.62	-16.65
13	1.03	35.41	27.24	-23.07	38.14	32.69	38.14	-14.29	13.62	13.62	.00
14	.85	46.31	43.58	-5.90	38.14	40.86	38.14	7.13	19.07	16.34	-14.32
15	-1.70	35.41	38.14	7.71	46.31	49.03	46.31	5.87	21.79	21.79	.00
16	-2.16	35.41	32.69	-7.68	35.41	32.69	35.41	-7.68	13.62	16.34	19.97
17	-10.48	49.03	43.58	-11.12	43.58	38.14	43.58	-12.48	16.34	13.62	-16.65
18	1.53	43.58	38.14	-12.48	43.58	43.58	43.58	.00	27.24	16.34	-40.01
19	-.53	51.76	46.31	-10.53	43.58	46.31	43.58	6.26	24.52	21.79	-11.13
20	.50	43.58	40.86	-6.24	35.41	29.96	35.41	-15.39	16.34	16.34	.00

ID	postLP	pcLP	preKE	postKE	pcKE	preKF	postKF	pcKF	preBAP	postBAP	pcBAP
1	113.50	13.64	43.58	40.86	-6.24	54.48	49.03	-10.00	13.38	14.97	11.88
7	81.72	5.88	40.86	43.58	6.66	46.31	46.31	.00	33.26	42.24	27.03
8	77.18	30.77	19.07	27.24	42.84	24.52	32.69	33.32	23.91	26.63	11.37
9	81.72	5.88	40.86	40.86	.00	38.14	40.86	7.13	24.38	31.00	27.14
10	68.10	.00	38.14	35.41	-7.16	49.03	49.03	.00	38.10	36.45	-4.32
11	136.20	25.00	59.93	59.93	.00	49.03	59.93	22.23	19.56	26.07	33.31
17	95.34	.00	46.31	49.03	5.87	54.48	57.20	4.99	22.09	20.73	-6.15
18	136.20	7.14	76.27	79.00	3.58	54.48	51.76	-4.99	33.68	37.02	9.91
19	72.64	23.08	27.24	24.52	-9.99	38.14	40.86	7.13	26.49	23.77	-10.27
20	122.58	-3.57	35.41	35.41	.00	35.41	43.58	23.07	28.92	27.83	-3.77
22	104.42	4.55	49.03	49.03	.00	46.31	59.93	29.41	29.61	25.95	-12.36
23	72.64	-15.79	32.69	32.69	.00	49.03	43.58	-11.12	18.34	24.37	32.83
25	131.66	45.00	54.48	57.20	4.99	54.48	54.48	.00	28.42	29.26	2.96
26	118.04	.00	40.86	43.58	6.66	38.14	43.58	14.26	20.47	23.18	13.27
28	95.34	40.00	32.69	35.41	8.32	27.24	32.69	20.01	25.40	22.44	-11.67
29	68.10	.00	49.03	46.31	-5.55	46.31	46.31	.00	13.32	16.47	23.68
1	90.80	-23.08	46.31	43.58	-5.90	49.03	43.58	-11.12	22.27	20.35	-8.62
2	90.80	-9.09	46.31	49.03	5.87	54.48	59.93	10.00	15.77	17.22	9.20
3	90.80	-23.08	43.58	43.58	.00	51.76	49.03	-5.27	49.70	52.77	6.18
4	136.20	7.14	29.96	40.86	36.38	46.31	54.48	17.64	18.43	16.47	-10.67
6	90.80	-4.76	46.31	49.03	5.87	49.03	49.03	.00	16.42	18.53	12.83
7	127.12	-17.65	49.03	49.03	.00	49.03	38.14	-22.21	22.60	19.14	-15.34
8	99.88	-8.33	49.03	49.03	.00	51.76	43.58	-15.80	24.10	21.20	-12.06
9	72.64	.00	46.31	46.31	.00	49.03	43.58	-11.12	33.63	32.08	-4.61
11	77.18	13.33	51.76	57.20	10.51	54.48	49.03	-10.00	29.31	22.65	-22.73
12	99.88	10.00	43.58	40.86	-6.24	32.69	43.58	33.31	37.16	32.83	-11.64
13	90.80	-23.08	35.41	38.14	7.71	49.03	49.03	.00	34.71	29.36	-15.41
14	99.88	-4.35	46.31	43.58	-5.90	49.03	49.03	.00	26.54	21.85	-17.68
15	99.88	4.76	46.31	46.31	.00	51.76	49.03	-5.27	35.00	30.30	-13.44
16	81.72	.00	29.96	29.96	.00	35.41	32.69	-7.68	41.95	41.80	-.36
17	90.80	-16.67	57.20	54.48	-4.76	51.76	51.76	.00	36.00	32.63	-9.36
18	108.96	-11.11	62.65	54.48	-13.04	57.20	59.93	4.77	31.59	32.58	3.13
19	108.96	-4.00	57.20	54.48	-4.76	49.03	49.03	.00	13.91	13.17	-5.31
20	108.96	-7.69	21.79	32.69	50.02	32.69	40.86	24.99	35.40	31.09	-12.17

ID	stT4CBMD	pcT4CBMD	preT4TBMD	stT4TBMD	pcT4TBMD	preT38BMD	stT38BMD	pcT38BMD	preT38CBMD	ostT38CBMD
1	695.0	1.91	256.1	254.2	-.74	937.90	938.20	.03	1183.70	1184.50
7	636.2	1.82	214.7	214.7	.00	947.50	936.40	-1.17	1155.50	1147.80
8	696.0	.43	202.5	204.6	1.04	920.00	919.10	-.10	1189.80	1187.50
9	699.5	3.23	198.4	199.6	.60	866.30	869.20	.33	1180.90	1180.40
10	689.0	1.58	182.0	181.2	-.44	983.20	986.10	.29	1212.80	1207.20
11	725.5	-6.74	258.6	262.0	1.31	1003.30	1001.20	-.21	1174.40	1172.80
17	677.4	4.14	218.9	219.4	.23	862.30	859.40	-.34	1209.80	1204.40
18	700.3	-.45	256.1	252.0	-1.60	924.10	923.20	-.10	1142.60	1141.90
19	686.9	-.42	261.4	261.3	-.04	991.60	981.70	-1.00	1216.80	1199.00
20	673.3	.01	253.6	254.4	.32	956.60	951.20	-.56	1204.80	1200.20
22	711.7	-1.14	267.8	270.5	1.01	973.10	971.30	-.18	1213.90	1213.70
23	646.2	-1.97	232.6	234.5	.82	851.50	852.00	.06	1158.60	1161.40
25	683.2	-1.41	236.0	234.2	-.76	1019.70	1017.00	-.26	1221.80	1218.80
26	729.3	1.60	261.6	263.6	.76	884.90	877.70	-.81	1174.70	1171.70
28	697.5	2.65	220.3	217.0	-1.50	929.80	933.30	.38	1194.30	1198.30
29	672.0	-1.12	199.1	201.6	1.26	959.10	959.70	.06	1199.40	1202.50
1	638.9	-1.33	193.4	194.8	.72	933.60	932.40	-.13	1193.20	1188.90
2	689.9	-2.57	245.0	240.5	-1.84	985.00	993.70	.88	1176.50	1187.40
3	662.1	11.82	269.3	255.1	-5.27	827.80	835.90	.98	1155.30	1155.70
4	806.9	-2.68	265.7	267.3	.60	949.70	955.20	.58	1226.70	1234.80
6	741.6	5.09	200.5	202.7	1.10	871.70	867.60	-.47	1117.80	1111.20
7	755.5	2.21	270.0	268.4	-.59	959.00	963.10	.43	1211.50	1216.50
8	770.0	-.99	238.6	241.1	1.05	904.90	907.60	.30	1184.80	1189.80
9	760.9	3.86	246.6	243.2	-1.38	942.40	934.40	-.85	1200.80	1188.90
11	731.2	1.78	222.6	220.3	-1.03	1007.00	997.70	-.92	1173.00	1161.80
12	706.0	-2.08	238.7	234.3	-1.84	829.80	827.10	-.33	1191.20	1191.10
13	733.3	-1.42	186.0	189.2	1.72	849.00	850.60	.19	1199.60	1202.40
14	721.7	1.15	280.6	284.2	1.28	1042.90	1043.70	.08	1228.00	1225.90
15	720.5	.13	214.4	213.4	-.47	968.20	966.30	-.20	1199.80	1201.90
16	659.8	-3.35	253.1	257.8	1.86	926.60	929.50	.31	1173.80	1177.80
17	725.9	.65	219.3	218.3	-.46	853.90	853.70	-.02	1131.20	1126.50
18	729.7	-3.71	252.3	256.0	1.47	895.40	905.40	1.12	1159.00	1164.40
19	753.2	-.91	214.8	219.5	2.19	858.60	860.70	.24	1195.40	1196.80
20	671.5	-.75	249.3	247.5	-.72	941.20	932.40	-.93	1169.20	1169.50

ID	preT38TBMD	stT38TBMD	pcT38TBMD	preT38SSI	stT38SSI	pcT38SSI	preT66BMD	stT66BMD	pcT66BMD	preT66CBMD
1	182.40	186.00	1.97	1644.35	1622.92	-1.30	646.80	648.00	.19	1139.30
7	199.80	203.10	1.65	1396.57	1387.35	-.66	696.70	691.10	-.80	1124.20
8	128.70	136.80	6.29	1348.19	1341.59	-.49	704.10	700.60	-.50	1150.70
9	198.00	202.60	2.32	1380.54	1372.24	-.60	568.50	566.60	-.33	1145.00
10	163.10	169.50	3.92	1193.76	1205.65	1.00	632.80	633.50	.11	1162.50
11	263.10	263.90	.30	1390.22	1367.58	-1.63	572.60	591.20	3.25	1111.10
17	84.60	82.40	-2.60	1450.94	1426.04	-1.72	548.00	542.80	-.95	1137.90
18	228.40	224.60	-1.66	1985.99	1999.96	.70	672.10	668.40	-.55	1070.40
19	192.20	172.50	-10.25	1223.23	1275.47	4.27	806.30	799.00	-.91	1169.90
20	215.50	207.60	-3.67	1062.86	1051.19	-1.10	669.90	670.90	.15	1144.80
22	93.00	88.20	-5.16	1542.10	1566.16	1.56	667.10	670.10	.45	1161.90
23	149.10	155.30	4.16	1595.67	1585.32	-.65	699.10	702.70	.51	1133.00
25	106.80	107.90	1.03	1439.07	1442.68	.25	686.10	683.60	-.36	1157.10
26	239.50	237.10	-1.00	1514.69	1521.34	.44	660.50	656.60	-.59	1121.10
28	186.90	183.30	-1.93	1268.59	1247.42	-1.67	697.10	688.70	-1.20	1165.90
29	148.30	152.90	3.10	1768.02	1744.31	-1.34	571.30	571.20	-.02	1169.30
1	138.90	129.10	-7.06	1577.43	1474.02	-6.56	555.90	563.40	1.35	1140.70
2	183.00	207.60	13.44	1515.25	1476.52	-2.56	704.60	720.20	2.21	1128.90
3	189.30	195.90	3.49	1415.74	1421.48	.41	700.80	680.20	-2.94	1122.20
4	93.10	93.50	.43	1459.43	1403.04	-3.86	757.60	761.60	.53	1181.90
6	232.10	229.80	-.99	963.46	1010.76	4.91	548.30	521.40	-4.91	1048.30
7	100.30	98.50	-1.79	1509.36	1525.70	1.08	686.20	684.40	-.26	1150.10
8	196.20	190.50	-2.91	1453.97	1473.52	1.34	640.30	638.90	-.22	1124.40
9	154.30	150.50	-2.46	1580.01	1609.94	1.89	662.80	658.20	-.69	1149.70
11	140.80	154.70	9.87	1404.78	1410.71	.42	704.40	729.50	3.56	1103.70
12	153.20	153.40	.13	1469.43	1509.05	2.70	636.10	634.70	-.22	1167.70
13	93.40	90.20	-3.43	1369.07	1370.78	.12	600.60	607.00	1.07	1155.80
14	126.70	131.10	3.47	1294.38	1326.43	2.48	747.90	733.00	-1.99	1153.90
15	100.20	103.40	3.19	1591.13	1543.10	-3.02	669.50	672.50	.45	1155.00
16	155.90	165.00	5.84	1136.94	1106.05	-2.72	679.40	680.60	.18	1121.60
17	193.40	184.30	-4.71	1604.30	1604.05	-.02	676.40	669.70	-.99	1083.80
18	174.30	172.00	-1.32	1398.96	1437.41	2.75	639.70	658.20	2.89	1123.00
19	106.50	102.80	-3.47	1710.13	1665.69	-2.60	592.00	611.70	3.33	1132.80
20	109.70	109.50	-.18	1509.95	1493.77	-1.07	660.80	661.00	.03	1131.50

ID	pcT66CBMD	preT66TBMD	stT66TBMD	pcT66TBMD	preT66SSI	stT66SSI	pcT66SSI	preF4BMD	postF4BMD	F4bmdcov
1	-.52	157.20	149.20	-5.09	2512.33	2487.50	-.99	412.90	419.90	412.90
7	-.84	147.90	152.80	3.31	2044.48	2044.79	.02	288.10	302.00	288.10
8	-.47	107.10	110.20	2.89	1983.54	1970.75	-.64	368.10	407.00	368.10
9	.18	138.30	134.70	-2.60	2065.83	2081.66	.77			
10	-.25	133.80	126.00	-5.83	1724.04	1698.41	-1.49	345.80	247.00	345.80
11	-.62	171.50	174.40	1.69	2660.10	2634.90	-.95	395.50	375.50	395.50
17	-.53	111.00	107.80	-2.88	2126.68	2095.47	-1.47	329.80	399.30	329.80
18	-.71	218.30	218.30	.00	2866.58	2842.56	-.84	443.00	455.70	443.00
19	-.50	183.20	182.70	-.27	1957.38	1992.03	1.77	377.00	340.50	377.00
20	-.19	139.00	138.30	-.50	1605.68	1652.71	2.93	326.00	399.40	326.00
22	-.07	125.40	124.30	-.88	2381.27	2361.14	-.85	349.10	354.50	349.10
23	-.26	124.40	119.00	-4.34	2403.21	2360.20	-1.79	429.00	606.20	429.00
25	.08	130.40	130.30	-.08	2180.11	2211.51	1.44	275.40	333.60	275.40
26	-.62	183.40	182.70	-.38	2235.35	2238.51	.14	345.10	346.60	345.10
28	-.39	143.10	143.10	.00	1922.80	1907.40	-.80	458.30	436.40	458.30
29	.20	97.40	93.50	-4.00	2955.17	2934.36	-.70	265.40	274.90	265.40
1	.00	99.70	102.10	2.41	2501.69	2424.63	-3.08	228.60	222.40	228.60
2	.38	195.80	194.30	-.77	2292.94	2228.97	-2.79	384.30	371.50	384.30
3	-.17	184.60	173.00	-6.28	1899.19	1939.40	2.12	421.60	488.30	421.60
4	-.52	144.30	134.80	-6.58	2303.17	2248.25	-2.38	499.40	453.50	499.40
6	-.03	127.40	125.60	-1.41	1590.64	1643.74	3.34	252.30	428.70	252.30
7	.32	156.60	158.40	1.15	2412.02	2440.99	1.20	388.90	415.40	388.90
8	-.18	165.40	163.60	-1.09	2022.74	1978.03	-2.21	348.70	341.50	348.70
9	-.20	146.20	138.70	-5.13	2303.65	2262.40	-1.79	398.50	350.30	398.50
11	.63	100.10	121.60	21.48	2057.13	2134.24	3.75	266.50	282.80	266.50
12	-.27	139.90	138.30	-1.14	2383.40	2374.94	-.35	374.80	420.30	374.80
13	-.22	86.90	89.60	3.11	2193.98	2190.26	-.17	346.30	379.70	346.30
14	-.71	176.90	176.90	.00	1943.06	1943.98	.05	477.00	370.60	477.00
15	.20	125.60	135.70	8.04	2353.62	2322.65	-1.32	350.00	396.50	350.00
16	-.62	186.20	179.70	-3.49	1683.99	1690.06	.36	97.62	103.45	97.62
17	-.67	197.80	194.40	-1.72	2251.84	2289.99	1.69	94.56	89.89	94.56
18	.45	152.30	165.80	8.86	1973.12	1970.43	-.14	92.69	96.90	92.69
19	.87	111.90	108.60	-2.95	2444.54	2454.11	.39	85.64	88.02	85.64
20	-.80	130.20	132.30	1.61	2235.85	2236.85	.04	86.33	88.51	86.33

ID	preF4CBMD	stF4CBMD	pcF4CBMD	preF4TBMD	stF4TBMD	pcF4TBMD	preF66BMD	stF66BMD	pcF66BMD	preF66CBMD
1	822.10	818.40	-.45	248.70	248.20	-.20	851.50	868.80	2.03	1175.60
7	655.50	673.00	2.67	216.60	222.60	2.77	875.10	862.50	-1.44	1150.30
8	858.30	947.30	10.37	140.90	109.60	-22.21	928.30	894.60	-3.63	1196.40
9							799.50	871.70	9.03	1131.90
10	812.80	638.80	-21.41	181.30	198.90	9.71	632.30	743.80	17.63	1122.70
11	797.40	767.70	-3.72	235.00	238.40	1.45	688.20	714.10	3.76	1088.70
17	754.40	879.60	16.60	207.10	170.60	-17.62	694.80	657.20	-5.41	1150.50
18	815.10	839.40	2.98	244.50	236.00	-3.48	804.60	805.10	.06	1127.40
19	773.70	697.10	-9.90	242.40	253.80	4.70	795.10	847.70	6.62	1125.70
20	662.20	777.60	17.43	256.60	238.70	-6.98	801.80	729.20	-9.05	1145.10
22	760.80	769.50	1.14	216.50	225.00	3.93	751.20	759.30	1.08	1158.00
23	815.60	1013.20	24.23	229.20	185.40	-19.11	729.70	571.00	-21.75	1127.70
25	676.80	743.20	9.81	214.10	210.60	-1.63	1003.30	990.20	-1.31	1211.00
26	691.60	719.60	4.05	261.00	247.90	-5.02	711.60	668.00	-6.13	1128.30
28	900.70	878.40	-2.48	184.80	181.10	-2.00	929.70	935.90	.67	1190.90
29	778.90	807.00	3.61	142.40	136.40	-4.21	587.90	580.80	-1.21	1153.40
1	650.60	651.50	.14	187.20	159.90	-14.58	629.30	634.90	.89	1119.70
2	800.50	779.80	-2.59	223.20	233.90	4.79	762.40	774.20	1.55	1089.70
3	842.90	904.70	7.33	214.90	206.90	-3.72	623.00	610.40	-2.02	1137.50
4	862.40	815.40	-5.45	282.10	286.80	1.67	945.70	937.90	-.82	1206.80
6	671.10	842.40	25.53	216.30	179.70	-16.92	590.70	481.10	-18.55	1035.90
7	798.80	859.40	7.59	231.50	216.70	-6.39	764.50	757.10	-.97	1161.20
8	749.60	740.70	-1.19	223.40	222.10	-.58	652.60	643.30	-1.43	1119.00
9	785.40	728.30	-7.27	255.00	259.50	1.76	667.80	662.50	-.79	1139.00
11	707.70	736.10	4.01	180.20	179.50	-.39	871.40	872.20	.09	1162.90
12	744.00	803.40	7.98	265.50	263.80	-.64	760.40	698.90	-8.09	1162.80
13	858.20	869.50	1.32	149.40	155.00	3.75	551.00	560.90	1.80	1094.80
14	909.70	775.90	-14.71	245.20	234.20	-4.49	804.60	948.40	17.87	1106.40
15	829.00	885.30	6.79	199.30	190.80	-4.26	670.50	617.40	-7.92	1138.60
16	865.20	849.90	-1.77	310.10	296.10	-4.51	723.00	722.90	-.01	1071.90
17	680.00	877.30	29.01	195.50	144.80	-25.93	732.00	660.80	-9.73	1132.00
18	876.40	822.80	-6.12	181.50	188.30	3.75	792.20	882.90	11.45	1106.10
19	785.90	795.90	1.27	190.70	186.30	-2.31	733.30	733.70	.05	1149.80
20	926.10	780.50	-15.72	180.90	220.50	21.89	750.30	796.10	6.10	1101.80

ID	pcF66CBMD	preF66TBMD	stF66TBMD	pcF66TBMD	preF66SSI	stF66SSI	pcF66SSI	preT4TOT_CNT	stT4TOT_CNT
1	.80	208.00	220.20	2.03	480.10	449.47	-6.38	321.39	318.07
7	-2.04	297.50	280.10	-1.44	358.41	341.76	-4.65	270.04	272.68
8	-1.39	145.50	147.40	-3.63	221.83	248.24	11.91	277.67	277.23
9	3.41	200.30	163.70	9.03	170.05	173.61	2.09	252.32	248.03
10	2.97	138.40	113.00	17.63	299.72	279.07	-6.89	201.39	204.93
11	-1.34	287.60	277.60	3.76	398.54	417.47	4.75	298.14	314.46
17	-.76	147.40	138.00	-5.41	299.40	294.83	-1.53	278.30	275.21
18	-.55	167.70	162.80	.06	251.69	241.12	-4.20	361.43	330.98
19	1.74	267.30	179.50	6.62	334.47	329.47	-1.49	252.83	260.62
20	-3.57	199.10	231.20	-9.05	259.75	293.24	12.89	236.70	236.24
22	.76	99.20	104.00	1.08	387.41	388.01	.15	304.44	322.70
23	-4.29	148.80	182.90	-21.75	299.05	295.96	-1.03	258.41	264.69
25	-1.13	157.50	158.80	-1.31	255.31	267.88	4.92	276.69	261.25
26	-3.49	185.80	208.90	-6.13	214.44	221.03	3.07	275.68	266.93
28	.97	124.40	138.00	.67	229.95	231.64	.73	227.47	224.82
29	-.94	146.70	131.20	-1.21	308.56	325.35	5.44	239.09	242.31
1	.32	94.30	89.50	.89	335.06	312.70	-6.67	234.82	237.34
2	2.20	170.90	158.60	1.55	403.15	388.76	-3.57	331.16	335.67
3	-2.13	170.00	182.50	-2.02	235.29	225.83	-4.02	297.84	255.83
4	-.25	117.90	156.60	-.82	267.56	267.98	.16	273.35	275.52
6	-1.24	217.80	200.30	-18.55	242.60	229.93	-5.22	206.74	203.82
7	-.16	148.00	133.30	-.97	340.01	338.70	-.39	276.51	275.67
8	-1.94	126.70	129.80	-1.43	250.14	251.99	.74	231.60	235.18
9	.01	153.30	140.20	-.79	387.50	393.48	1.54	270.95	267.45
11	1.05	167.80	176.80	.09	191.70	184.27	-3.88	274.66	273.88
12	-2.92	163.50	137.60	-8.09	269.50	288.29	6.97	285.61	288.52
13	-.23	118.10	116.80	1.80	304.57	301.15	-1.12	229.05	229.52
14	4.63	286.80	198.10	17.87	346.99	337.40	-2.76	271.30	269.75
15	-3.30	118.30	173.40	-7.92	402.37	397.39	-1.24	266.08	264.22
16	.53	296.30	283.80	-.01	242.86	254.46	4.78	249.34	256.79
17	-1.90	169.50	142.00	-9.73	316.04	323.22	2.27	240.63	243.46
18	4.23	171.80	145.50	11.45	342.57	256.12	-25.24	246.83	258.32
19	-.27	152.10	149.40	.05	247.01	276.72	12.03	277.15	279.39
20	3.95	211.60	152.60	6.10	267.66	270.39	1.02	243.15	244.04

ID	preT4Trab CNT	stT4Trab CNT	T4Trab CNT	preT4TOT A	stT4TOT A	pcT4TOT A	preT4Trab A	postT4Trab A
1	231.03	220.66	-4.49	1058.08	1029.92	-2.66	902.08	868.16
7	211.57	212.40	.39	1114.08	1120.48	.57	985.60	989.28
8	190.72	187.32	-1.78	1097.76	1075.20	-2.06	941.60	915.52
9	176.58	162.83	-7.79	1033.28	967.20	-6.40	889.92	815.68
10	130.07	131.27	.92	842.08	856.48	1.71	714.88	724.32
11	169.08	202.59	19.82	838.40	949.12	13.21	653.92	773.12
17	204.24	192.59	-5.70	1074.40	1026.08	-4.50	933.12	877.92
18	249.96	231.85	-7.25	1160.64	1086.72	-6.37	976.16	920.16
19	172.88	175.71	1.64	790.56	807.20	2.10	661.44	672.32
20	175.26	174.99	-.15	804.96	800.00	-.62	691.04	687.84
22	198.99	212.36	6.72	905.92	956.16	5.55	743.04	784.96
23	183.64	196.98	7.26	925.12	968.00	4.64	789.44	840.00
25	193.05	184.36	-4.50	964.32	924.48	-4.13	817.92	787.20
26	193.52	184.07	-4.88	880.64	834.08	-5.29	739.68	698.24
28	162.17	154.12	-4.96	861.28	841.44	-2.30	736.00	710.08
29	167.87	172.95	3.03	984.00	995.20	1.14	843.36	857.92
1	176.63	184.53	4.47	1041.76	1071.20	2.83	913.44	947.36
2	220.93	233.34	5.62	1082.72	1147.84	6.01	901.76	970.40
3	246.94	181.31	-26.58	1029.92	838.56	-18.58	916.80	710.72
4	136.02	141.39	3.95	694.24	709.28	2.17	512.00	528.96
6	144.05	115.51	-19.81	837.60	704.32	-15.91	718.40	569.92
7	185.79	183.11	-1.44	835.20	830.08	-.61	688.00	682.24
8	136.57	137.39	.60	711.68	714.24	.36	572.32	569.92
9	172.01	170.48	-.89	855.20	854.56	-.07	697.44	701.12
11	170.30	169.25	-.62	930.88	936.32	.58	764.96	768.32
12	205.10	203.20	-.93	1005.28	1017.28	1.19	859.36	867.20
13	138.97	141.63	1.91	894.72	894.72	.00	747.20	748.64
14	187.76	180.47	-3.88	804.80	772.80	-3.98	669.12	635.04
15	184.32	182.25	-1.12	1006.56	1001.60	-.49	859.84	854.08
16	173.99	187.82	7.95	812.64	849.12	4.49	687.52	728.64
17	149.94	149.07	-.58	828.00	832.00	.48	683.68	682.72
18	146.43	154.84	5.74	728.96	762.08	4.54	580.48	604.96
19	153.76	167.30	8.81	900.32	939.36	4.34	715.68	762.08
20	177.18	183.08	3.33	828.32	854.08	3.11	710.72	739.84

ID	preT4Cor CNT	stT4Cor CNT	cT4Cor CNT	preT4Cor A	stT4Cor A	pcT4Cor A	preT4C Thk	stT4C Thk	pcT4C Thk
1	75.29	82.51	9.59	110.40	118.72	7.54	.98	1.08	10.20
7	33.99	34.61	1.82	54.40	54.40	.00	.47	.46	-2.13
8	71.52	74.28	3.86	103.20	106.72	3.41	.90	.94	4.44
9	56.59	70.17	24.00	83.52	100.32	20.11	.75	.94	25.33
10	59.69	61.85	3.62	88.00	89.76	2.00	.88	.89	1.14
11	122.47	105.41	-13.93	157.44	145.28	-7.72	1.61	1.39	-13.66
17	56.10	68.07	21.34	86.24	100.48	16.51	.76	.91	19.74
18	99.28	95.24	-4.07	141.12	136.00	-3.63	1.21	1.20	-.83
19	87.20	85.95	-1.43	126.40	125.12	-1.01	1.32	1.30	-1.52
20	54.07	55.27	2.22	80.32	82.08	2.19	.82	.84	2.44
22	111.50	109.20	-2.06	154.88	153.44	-.93	1.52	1.46	-3.95
23	71.30	61.52	-13.72	108.16	95.20	-11.98	1.03	.89	-13.59
25	71.40	75.75	6.09	103.04	110.88	7.61	.96	1.06	10.42
26	81.66	82.50	1.03	113.76	113.12	-.56	1.12	1.15	2.68
28	61.87	66.07	6.79	91.04	94.72	4.04	.90	.95	5.56
29	74.16	71.18	-4.02	109.12	105.92	-2.93	1.01	.97	-3.96
1	48.07	40.17	-16.43	74.24	62.88	-15.30	.66	.55	-16.67
2	99.47	85.77	-13.77	140.48	124.32	-11.50	1.25	1.07	-14.40
3	35.43	67.69	91.05	59.84	102.24	70.86	.53	1.03	94.34
4	133.32	129.23	-3.07	160.80	160.16	-.40	1.84	1.81	-1.63
6	51.94	83.89	61.51	73.60	113.12	53.70	.73	1.26	72.60
7	85.87	88.97	3.61	116.16	117.76	1.38	1.18	1.20	1.69
8	96.56	95.98	-.60	124.16	124.64	.39	1.38	1.38	.00
9	101.39	98.13	-3.22	138.40	128.96	-6.82	1.39	1.30	-6.47
11	95.64	92.89	-2.88	133.12	127.04	-4.57	1.28	1.21	-5.47
12	70.37	71.84	2.09	97.60	101.76	4.26	.89	.92	3.37
13	79.27	77.09	-2.75	106.56	105.12	-1.35	1.04	1.02	-1.92
14	82.42	92.03	11.66	115.52	127.52	10.39	1.19	1.35	13.45
15	78.75	78.97	.28	109.44	109.60	.15	1.00	1.01	1.00
16	65.21	56.37	-13.56	95.52	85.44	-10.55	.98	.85	-13.27
17	82.73	86.88	5.02	114.72	119.68	4.32	1.17	1.22	4.27
18	95.18	98.66	3.66	125.60	135.20	7.64	1.37	1.45	5.84
19	115.54	101.71	-11.97	152.00	135.04	-11.16	1.50	1.29	-14.00
20	50.12	42.01	-16.18	74.08	62.56	-15.55	.74	.62	-16.22

ID	stT4Peri_C	pcT4Peri_C	preT38TOT_CNT	stT38TOT_CNT	pcT38TOT_CNT	preT38Trab_CNT	stT38Trab_CNT
1	113.76	-1.34	365.85	367.93	.57	17.46	17.95
7	118.66	.29	320.33	316.13	-1.31	14.61	15.24
8	116.24	-1.03	312.96	313.24	.09	11.12	11.91
9	110.25	-3.25	303.57	301.77	-.59	22.08	22.20
10	103.74	.85	290.39	291.90	.52	10.49	10.66
11	109.21	6.40	347.21	343.12	-1.18	16.88	16.89
17	113.55	-2.28	311.39	309.38	-.65	9.43	9.11
18	116.86	-3.24	408.84	408.43	-.10	24.08	23.64
19	100.72	1.05	315.88	322.64	2.14	13.41	11.90
20	100.27	-.31	273.97	271.50	-.90	15.48	14.84
22	109.62	2.74	366.65	363.65	-.82	7.53	7.11
23	110.29	2.29	333.24	333.18	-.02	17.68	18.66
25	107.78	-2.09	341.16	342.19	.30	6.48	6.59
26	102.38	-2.68	336.54	335.06	-.44	28.20	28.46
28	102.83	-1.15	284.01	286.56	.90	14.95	14.67
29	111.83	.57	377.18	376.95	-.06	13.33	13.88
1	116.02	1.40	344.31	344.63	.09	12.60	11.55
2	120.10	2.97	360.58	356.61	-1.10	12.85	14.71
3	102.65	-9.77	297.87	303.34	1.84	23.05	23.67
4	94.41	1.08	322.76	320.64	-.66	7.73	7.69
6	94.08	-8.30	249.52	253.77	1.70	18.42	18.50
7	102.13	-.31	344.31	347.32	.87	8.18	8.05
8	94.74	.18	320.83	322.97	.67	19.69	19.15
9	103.63	-.04	341.07	343.55	.73	13.77	13.53
11	108.47	.29	329.65	329.15	-.15	7.41	8.32
12	113.06	.59	310.00	308.73	-.41	19.85	20.05
13	106.04	.00	282.00	280.48	-.54	9.84	9.41
14	98.55	-2.01	338.41	341.67	.96	6.89	7.13
15	112.19	-.25	354.89	353.89	-.28	7.73	8.13
16	103.30	2.23	279.77	277.51	-.81	11.40	12.06
17	102.25	.24	337.60	338.87	.38	22.53	21.03
18	97.86	2.25	305.57	311.88	2.06	15.89	15.44
19	108.65	2.14	340.40	340.03	-.11	13.05	12.47
20	103.60	1.55	345.01	340.72	-1.24	8.65	8.95

ID	preT38TOT_A	stT38TOT_A	pcT38TOT_A	preT38Trab_A	stT38Trab_A	pcT38Trab_A	preT38Cor_CNT	ID
1	390.08	392.16	.53	95.68	96.48	.84	348.28	1
7	338.08	337.60	-.14	73.12	75.04	2.63	305.05	7
8	340.16	340.80	.19	86.40	87.04	.74	301.73	8
9	350.40	347.20	-.91	111.52	109.60	-1.72	280.78	9
10	295.36	296.00	.22	64.32	62.88	-2.24	279.61	10
11	346.08	342.72	-.97	64.16	64.00	-.25	329.40	11
17	361.12	360.00	-.31	111.52	110.56	-.86	301.96	17
18	442.40	442.40	.00	105.44	105.28	-.15	384.46	18
19	318.56	328.64	3.16	69.76	68.96	-1.15	302.17	19
20	286.40	285.44	-.34	71.84	71.52	-.45	258.49	20
22	376.80	374.40	-.64	80.96	80.64	-.40	359.12	22
23	391.36	391.04	-.08	118.56	120.16	1.35	314.96	23
25	334.56	336.48	.57	60.64	61.12	.79	334.68	25
26	380.32	381.76	.38	117.76	120.00	1.90	308.23	26
28	305.44	307.04	.52	80.00	80.00	.00	268.85	28
29	393.28	392.80	-.12	89.92	90.72	.89	363.85	29
1	368.80	369.60	.22	90.72	89.44	-1.41	331.60	1
2	366.08	358.88	-1.97	70.24	70.88	.91	347.31	2
3	359.84	362.88	.84	121.76	120.80	-.79	274.50	3
4	339.84	335.68	-1.22	83.04	82.24	-.96	315.02	4
6	286.24	292.48	2.18	79.36	80.48	1.41	230.90	6
7	359.04	360.64	.45	81.60	81.76	.20	336.13	7
8	354.56	355.84	.36	100.32	100.48	.16	301.05	8
9	361.92	367.68	1.59	89.28	89.92	.72	327.19	9
11	327.36	329.92	.78	52.64	53.76	2.13	322.24	11
12	373.60	373.28	-.09	129.60	130.72	.86	289.51	12
13	332.16	329.76	-.72	105.28	104.32	-.91	272.17	13
14	324.48	327.36	.89	54.40	54.40	.00	331.46	14
15	366.56	366.24	-.09	77.12	78.56	1.87	347.08	15
16	301.92	298.56	-1.11	73.12	73.12	.00	268.18	16
17	395.36	396.96	.40	116.48	114.08	-2.06	314.56	17
18	341.28	344.48	.94	91.20	89.76	-1.58	289.48	18
19	396.48	395.04	-.36	122.56	121.28	-1.04	327.24	19
20	366.56	365.44	-.31	78.88	81.76	3.65	365.44	20

cT38Cor CNT	preT38Cor A	stT38Cor A	pcT38Cor A	preT38C Thk	postT38C Thk	pcT38C Thk	preT38Peri C
.40	294.24	295.20	.33	5.62	5.62	.00	70.01
-1.57	264.00	261.60	-.91	5.52	5.45	-1.27	65.18
-.13	253.60	253.76	.06	5.16	5.15	-.19	65.38
-.79	237.76	236.00	-.74	4.57	4.56	-.22	66.36
.51	230.56	232.80	.97	5.16	5.22	1.16	60.92
-1.22	280.48	277.44	-1.08	5.93	5.89	-.67	65.95
-.64	249.60	249.12	-.19	4.76	4.76	.00	67.36
.03	336.48	336.80	.10	6.06	6.07	.17	74.56
2.60	248.32	258.56	4.12	5.34	5.51	3.18	63.27
-.75	214.56	213.76	-.37	4.77	4.76	-.21	59.99
-.72	295.84	293.76	-.70	5.88	5.85	-.51	68.81
-.17	271.84	270.72	-.41	4.99	4.97	-.40	70.13
.27	273.92	275.36	.53	5.93	5.94	.17	64.84
-.56	262.40	261.60	-.30	4.88	4.84	-.82	69.13
1.05	225.12	226.72	.71	4.80	4.83	.63	61.95
-.27	303.36	301.76	-.53	5.84	5.80	-.68	70.30
.45	277.92	280.16	.81	5.46	5.51	.92	68.08
-1.59	295.20	287.84	-2.49	6.05	5.93	-1.98	67.83
1.85	237.60	241.92	1.82	4.47	4.54	1.57	67.25
-.66	256.80	253.44	-1.31	5.26	5.22	-.76	65.35
1.71	206.56	211.36	2.32	4.51	4.57	1.33	59.98
.93	277.44	278.88	.52	5.59	5.61	.36	67.17
.92	254.08	255.36	.50	4.97	4.99	.40	66.75
.81	272.48	277.44	1.82	5.40	5.46	1.11	67.44
-.43	274.72	276.16	.52	6.12	6.11	-.16	64.14
-.40	243.04	242.08	-.39	4.46	4.44	-.45	68.52
-.40	226.88	225.44	-.63	4.49	4.48	-.22	64.61
.90	269.92	272.80	1.07	6.00	6.04	.67	63.86
-.38	289.28	287.68	-.55	5.84	5.80	-.68	67.87
-1.06	228.48	225.28	-1.40	4.97	4.92	-1.01	61.60
.67	278.08	281.12	1.09	5.11	5.17	1.17	70.49
2.33	249.76	254.40	1.86	5.03	5.12	1.79	65.49
.06	273.76	273.60	-.06	4.98	5.00	.40	70.59
-9.21	287.68	283.68	-1.39	5.79	5.68	-1.90	67.87

ID	pcT38Peri C	preT66TOT CNT	postT66TOT CNT	pcT66TOT CNT	reT66Trab CNT	stT66Trab CNT	pcT66Trab CNT
1	.27	380.74	380.94	.05	46.31	43.17	-6.78
7	-.08	329.74	326.99	-.83	30.60	31.82	3.99
8	.09	332.43	330.59	-.55	21.63	22.33	3.24
9	-.47	306.56	305.69	-.28	42.68	41.62	-2.48
10	.11	291.99	292.33	.12	31.78	29.55	-7.02
11	-.49	371.22	372.58	.37	63.33	60.19	-4.96
17	-.15	308.92	308.46	-.15	35.88	35.19	-1.92
18	.00	421.64	418.82	-.67	63.61	63.54	-.11
19	1.56	367.93	368.82	.24	30.71	31.24	1.73
20	-.17	278.80	280.36	.56	27.31	27.16	-.55
22	-.32	370.60	371.06	.12	33.25	32.58	-2.02
23	-.04	375.64	374.51	-.30	28.75	26.80	-6.78
25	.29	347.64	348.60	.28	30.29	30.67	1.25
26	.19	354.89	357.22	.66	48.30	48.66	.75
28	.27	322.46	322.30	-.05	30.25	31.05	2.64
29	-.06	389.57	389.96	.10	37.04	35.53	-4.08
1	.10	343.17	343.53	.10	34.55	34.59	.12
2	-.99	378.69	377.52	-.31	47.82	44.73	-6.46
3	.42	322.14	321.61	-.16	38.01	37.70	-.82
4	-.61	381.20	376.52	-1.23	29.66	26.50	-10.65
6	1.08	259.23	256.92	-.89	32.62	35.24	8.03
7	.22	384.94	388.43	.91	40.97	42.24	3.10
8	.18	334.10	331.93	-.65	43.50	42.76	-1.70
9	.79	357.79	357.01	-.22	38.21	36.43	-4.66
11	.39	334.03	349.01	4.48	18.84	22.33	18.52
12	-.04	363.02	361.03	-.55	41.24	40.53	-1.72
13	-.37	322.21	321.25	-.30	24.20	24.35	.62
14	.44	342.35	338.71	-1.06	33.52	34.52	2.98
15	-.04	362.08	360.81	-.35	31.98	34.50	7.88
16	-.57	303.60	302.97	-.21	39.31	37.10	-5.62
17	.20	363.94	365.92	.54	48.44	48.75	.64
18	.00	321.27	320.55	-.22	38.01	39.31	3.42
19	-.18	351.60	353.32	.49	35.17	32.18	-8.50
20	-.15	350.39	348.99	-.40	32.44	32.48	.12

ID	stT66TOT A	pcT66TOT A	preT66Trab A	postT66Trab A	pcT66Trab A	preT66Cor CNT	pcT66Cor CNT
1	587.84	-.14	294.56	289.44	-1.74	333.59	1.06
7	473.12	-.03	206.88	208.16	.62	298.77	-1.31
8	471.84	-.07	201.92	202.56	.32	310.59	-.82
9	539.52	.06	308.48	309.12	.21	263.45	.11
10	461.44	.00	237.44	234.56	-1.21	260.02	.90
11	630.24	-2.79	369.28	345.12	-6.54	305.05	1.36
17	568.32	.82	323.36	326.56	.99	272.37	.14
18	626.56	-.13	291.36	291.04	-.11	355.53	-.70
19	461.60	1.16	167.68	171.04	2.00	336.57	.05
20	417.92	.42	196.48	196.32	-.08	251.49	.68
22	553.76	-.32	265.12	262.24	-1.09	337.24	.37
23	532.96	-.80	231.04	225.12	-2.56	346.78	.20
25	509.92	.63	232.32	235.36	1.31	317.14	.25
26	544.00	1.25	263.36	266.40	1.15	305.82	.55
28	468.00	1.18	211.36	216.96	2.65	291.39	-.20
29	682.72	.12	380.16	379.84	-.08	352.11	.51
1	609.76	-1.22	346.40	338.72	-2.22	308.09	.18
2	524.16	-2.47	244.16	230.24	-5.70	330.56	.59
3	472.80	2.85	205.92	217.92	5.83	283.32	-.55
4	494.40	-1.75	205.60	196.64	-4.36	351.35	-.41
6	492.80	4.23	256.00	280.64	9.62	225.43	-2.19
7	567.52	1.17	261.60	266.56	1.90	343.56	.38
8	519.52	-.43	263.04	261.28	-.67	290.18	-.68
9	542.40	.47	261.28	262.56	.49	318.78	.38
11	478.40	.88	188.16	183.68	-2.38	314.35	3.62
12	568.80	-.34	294.88	292.96	-.65	321.36	-.57
13	529.28	-1.34	278.56	271.84	-2.41	297.91	-.34
14	462.08	.94	189.44	195.20	3.04	307.78	-1.90
15	536.48	-.80	254.56	254.24	-.13	329.50	-1.09
16	445.12	-.39	211.04	206.40	-2.20	263.97	.60
17	546.40	1.55	244.96	250.72	2.35	312.31	1.03
18	487.04	-3.03	249.60	237.12	-5.00	282.63	-.82
19	577.60	-2.75	314.24	296.48	-5.65	315.92	1.62
20	528.00	-.42	249.12	245.44	-1.48	317.74	-.64

ID	reT66Cor A	ostT66Cor A	pcT66Cor A	reT66C Thk	postT66C Thk	pcT66C Thk	reT66Peri C	ostT66Peri C
1	292.80	297.44	1.58	3.98	4.07	2.26	86.01	85.95
7	265.76	264.48	-.48	4.15	4.12	-.72	77.12	77.11
8	269.92	268.96	-.36	4.24	4.22	-.47	77.03	77.00
9	230.08	229.92	-.07	3.18	3.18	.00	82.32	82.34
10	223.68	226.24	1.14	3.42	3.47	1.46	76.15	76.15
11	274.56	280.00	1.98	3.46	3.61	4.34	90.26	88.99
17	239.36	240.96	.67	3.24	3.24	.00	84.16	84.51
18	332.16	332.16	.00	4.44	4.44	.00	88.79	88.73
19	287.68	289.28	.56	4.73	4.72	-.21	75.73	76.16
20	219.68	221.60	.87	3.60	3.63	.83	72.32	72.47
22	290.24	291.52	.44	4.11	4.14	.73	83.55	83.42
23	306.08	307.52	.47	4.50	4.55	1.11	82.17	81.84
25	274.08	274.56	.18	4.10	4.09	-.24	79.80	80.05
26	272.80	276.00	1.17	3.90	3.92	.51	82.17	82.68
28	249.92	250.40	.19	3.91	3.88	-.77	76.24	76.69
29	301.12	302.08	.32	3.72	3.73	.27	92.57	92.63
1	270.08	270.56	.18	3.51	3.54	.85	88.07	87.54
2	292.80	293.44	.22	4.26	4.35	2.11	82.18	81.16
3	252.48	251.52	-.38	3.98	3.88	-2.51	76.00	77.08
4	297.28	297.60	.11	4.56	4.63	1.54	79.52	78.82
6	215.04	210.40	-2.16	3.21	3.04	-5.30	77.08	78.69
7	298.72	298.88	.05	4.23	4.19	-.95	83.96	84.45
8	258.08	256.80	-.50	3.73	3.72	-.27	80.97	80.80
9	277.28	278.88	.58	3.97	3.98	.25	82.36	82.56
11	284.80	293.28	2.98	4.52	4.66	3.10	77.20	77.54
12	275.20	274.40	-.29	3.78	3.78	.00	84.69	84.54
13	257.76	257.44	-.12	3.65	3.68	.82	82.11	81.55
14	266.72	263.52	-1.20	4.27	4.18	-2.11	75.84	76.20
15	285.28	281.60	-1.29	4.10	4.06	-.98	82.44	82.11
16	235.36	238.24	1.22	3.72	3.79	1.88	74.94	74.79
17	288.16	293.12	1.72	4.17	4.21	.96	82.23	82.86
18	251.68	248.48	-1.27	3.71	3.74	.81	79.44	78.23
19	278.88	280.96	.75	3.74	3.84	2.67	86.39	85.20
20	280.80	281.28	.17	4.08	4.10	.49	81.63	81.46

ID	pcT66Peri C	reF4TOT CNT	postF4TOT CNT	pcF4TOT CNT	reF4Trab CNT	F4Trab CNT	cF4Trab CNT	reF4TOT A
1	-.07	117.73	122.69	4.21	49.61	49.13	-.97	285.12
7	-.01	88.70	93.07	4.93	54.38	55.16	1.43	307.84
8	-.04	98.95	90.33	-8.71	25.03	14.93	-40.35	268.80
9	.02							
10	.00	79.61	78.18	-1.80	29.88	53.95	80.56	230.24
11	-1.41	119.16	119.03	-.11	49.70	54.74	10.14	301.28
17	.42	90.25	88.41	-2.04	43.18	24.60	-43.03	273.60
18	-.07	106.39	97.78	-8.09	37.75	31.99	-15.26	240.16
19	.57	98.61	93.61	-5.07	45.81	54.83	19.69	261.60
20	.21	73.13	74.07	1.29	46.67	30.21	-35.27	224.32
22	-.16	100.05	102.09	2.04	45.48	47.96	5.45	286.56
23	-.40	107.64	98.65	-8.35	37.15	14.15	-61.91	250.88
25	.31	102.85	100.67	-2.12	66.74	47.91	-28.21	373.44
26	.62	87.46	74.31	-15.04	51.75	40.85	-21.06	253.44
28	.59	91.89	91.54	-.38	22.21	23.36	5.18	200.48
29	.06	77.93	77.64	-.37	32.51	29.37	-9.66	293.60
1	-.60	91.92	80.35	-12.59	65.17	49.06	-24.72	402.08
2	-1.24	112.60	114.26	1.47	45.96	52.96	15.23	292.96
3	1.42	82.77	84.77	2.42	27.82	20.98	-24.59	196.32
4	-.88	111.87	111.46	-.37	39.27	47.64	21.31	224.00
6	2.09	85.21	79.50	-6.70	63.30	20.47	-67.66	337.76
7	.58	107.82	106.46	-1.26	45.49	37.40	-17.78	277.28
8	-.21	92.66	93.21	.59	44.26	45.87	3.64	265.76
9	.24	108.26	97.07	-10.34	49.21	56.25	14.31	271.68
11	.44	71.00	68.05	-4.15	38.75	34.03	-12.18	266.40
12	-.18	99.72	101.35	1.63	53.45	44.44	-16.86	266.08
13	-.68	85.93	96.77	12.61	25.63	26.17	2.11	248.16
14	.47	101.88	87.35	-14.26	32.52	39.99	22.97	213.60
15	-.40	110.04	114.08	3.67	45.89	37.15	-19.05	314.40
16	-.20	97.62	103.45	5.97	33.79	38.14	12.87	182.88
17	.77	94.56	89.89	-4.94	54.45	24.98	-54.12	343.04
18	-1.52	92.69	96.90	4.54	27.15	32.56	19.93	227.52
19	-1.38	85.64	88.02	2.78	34.57	33.72	-2.46	249.12
20	-.21	86.33	88.51	2.53	19.42	38.99	100.77	183.04

ID	postF4TOT A	pcF4TOT A	reF4Trab A	postF4Trab A	cF4Trab A	F4Cor CNT
1	292.16	2.47	199.52	197.92	-.80	73.00
7	308.16	.10	251.04	247.84	-1.27	34.72
8	221.92	-17.44	177.60	136.16	-23.33	75.39
9						
10	316.48	37.46	164.80	271.20	64.56	50.20
11	316.96	5.20	211.52	229.60	8.55	72.72
17	221.44	-19.06	208.48	144.16	-30.85	51.90
18	214.56	-10.66	154.40	135.52	-12.23	70.82
19	274.88	5.08	188.96	216.00	14.31	52.36
20	185.44	-17.33	181.92	126.56	-30.43	28.82
22	288.00	.50	210.08	213.12	1.45	55.88
23	162.72	-35.14	162.08	76.32	-52.91	73.60
25	301.76	-19.19	311.68	227.52	-27.00	34.00
26	214.40	-15.40	198.24	164.80	-16.87	38.29
28	209.76	4.63	120.16	128.96	7.32	71.05
29	282.40	-3.81	228.32	215.36	-5.68	54.71
1	361.28	-10.15	348.16	306.72	-11.90	27.59
2	307.52	4.97	205.92	226.40	9.95	67.24
3	173.60	-11.57	129.44	101.44	-21.63	58.26
4	245.76	9.71	139.20	166.08	19.31	76.16
6	185.44	-45.10	292.64	113.92	-61.07	17.18
7	256.32	-7.56	196.48	172.64	-12.13	64.42
8	272.96	2.71	198.08	206.56	4.28	50.13
9	277.12	2.00	192.96	216.80	12.35	60.57
11	240.64	-9.67	215.04	189.60	-11.83	39.52
12	241.12	-9.38	201.28	168.48	-16.30	57.26
13	254.88	2.71	171.52	168.80	-1.59	69.48
14	235.68	10.34	132.64	170.72	28.71	70.30
15	287.68	-8.50	230.24	194.72	-15.43	71.22
16	206.56	12.95	108.96	128.80	18.21	70.88
17	251.68	-26.63	278.56	172.48	-38.08	42.76
18	253.76	11.53	149.60	172.96	15.61	71.37
19	253.12	1.61	181.28	180.96	-.18	58.22
20	242.08	32.26	107.36	176.80	64.68	69.20

ID	preF4Cor_CNT	postF4Cor_CNT	pcF4Cor_CNT	preF4Cor_A	postF4Cor_A	pcF4Cor_A	preF4C_Thk	postF4C_Thk
1	73.00	74.51	2.07	88.80	91.04	2.52	1.62	1.64
7	34.72	40.06	15.38	52.96	59.52	12.39	.89	1.01
8	75.39	81.55	8.17	87.84	86.08	-2.00	1.66	1.83
9								
10	50.20	21.87	-56.43	61.76	34.24	-44.56	1.24	.56
11	72.72	67.68	-6.93	91.20	88.16	-3.33	1.62	1.51
17	51.90	64.46	24.20	68.80	73.28	6.51	1.26	1.53
18	70.82	75.61	6.76	86.88	90.08	3.68	1.76	1.97
19	52.36	45.17	-13.73	67.68	64.80	-4.26	1.27	1.18
20	28.82	45.16	56.70	43.52	58.08	33.46	.86	1.32
22	55.88	56.63	1.34	73.44	73.60	.22	1.31	1.31
23	73.60	86.89	18.06	90.24	85.76	-4.96	1.79	2.25
25	34.00	56.37	65.79	50.24	75.84	50.96	.76	1.32
26	38.29	42.25	10.34	55.36	58.72	6.07	1.04	1.22
28	71.05	69.85	-1.69	78.88	79.52	.81	1.77	1.73
29	54.71	57.72	5.50	70.24	71.52	1.82	1.24	1.29
1	27.59	29.08	5.40	42.40	44.64	5.28	.61	.68
2	67.24	64.13	-4.63	84.00	82.24	-2.10	1.50	1.43
3	58.26	66.73	14.54	69.12	73.76	6.71	1.54	1.80
4	76.16	70.97	-6.81	88.32	87.04	-1.45	1.87	1.74
6	17.18	61.87	260.13	25.60	73.44	186.88	.40	1.71
7	64.42	69.17	7.37	80.64	80.48	-.20	1.48	1.55
8	50.13	50.60	.94	66.88	68.32	2.15	1.24	1.25
9	60.57	48.94	-19.20	77.12	67.20	-12.86	1.43	1.22
11	39.52	42.75	8.17	55.84	58.08	4.01	1.02	1.13
12	57.26	65.43	14.27	76.96	81.44	5.82	1.44	1.63
13	69.48	72.20	3.91	80.96	83.04	2.57	1.59	1.61
14	70.30	57.97	-17.54	77.28	74.72	-3.31	1.66	1.50
15	71.22	82.16	15.36	85.92	92.80	8.01	1.48	1.69
16	70.88	68.53	-3.32	81.92	80.64	-1.56	1.96	1.78
17	42.76	67.66	58.23	62.88	77.12	22.65	1.01	1.50
18	71.37	67.93	-4.82	81.44	82.56	1.38	1.69	1.61
19	58.22	59.59	2.35	74.08	74.88	1.08	1.44	1.44
20	69.20	55.94	-19.16	74.72	71.68	-4.07	1.76	1.41

ID	pcF4C_Thk	preF4Peri_C	stF4Peri_C	pcF4Peri_C	preF66TOT_CNT	postF66TOT_CNT	pcF66TOT_CNT
1	1.23	59.86	60.59	1.22	142.78	141.23	-1.09
7	13.48	62.20	62.23	.05	122.80	121.17	-1.33
8	10.24	58.12	52.81	-9.14	87.34	91.18	4.40
9					67.03	70.01	4.45
10	-54.84	53.79	63.06	17.23	84.68	86.39	2.02
11	-6.79	61.53	63.11	2.57	114.96	116.08	.97
17	21.43	58.64	52.75	-10.04	87.60	85.18	-2.76
18	11.93	54.94	51.93	-5.48	85.35	85.53	.21
19	-7.09	57.34	58.77	2.49	107.37	106.06	-1.22
20	53.49	53.09	48.27	-9.08	91.22	91.83	.67
22	.00	60.01	60.16	.25	112.62	111.52	-.98
23	25.70	56.15	45.22	-19.47	91.88	85.88	-6.53
25	73.68	68.50	61.58	-10.10	101.77	104.09	2.28
26	17.31	56.43	51.91	-8.01	71.84	73.75	2.66
28	-2.26	50.19	51.34	2.29	87.47	87.30	-.19
29	4.03	60.74	59.57	-1.93	87.39	89.30	2.19
1	11.48	71.08	67.38	-5.21	88.00	88.38	.43
2	-4.67	60.68	62.16	2.44	113.69	111.85	-1.62
3	16.88	49.67	46.71	-5.96	72.17	68.56	-5.00
4	-6.95	53.06	55.57	4.73	96.54	96.34	-.21
6	327.50	65.15	48.27	-25.91	75.04	61.82	-17.62
7	4.73	59.03	56.75	-3.86	104.94	100.42	-4.31
8	.81	57.79	58.57	1.35	74.87	75.24	.49
9	-14.69	58.43	59.01	.99	104.81	103.99	-.78
11	10.78	57.86	54.99	-4.96	74.73	74.52	-.28
12	13.19	57.82	55.05	-4.79	90.03	89.46	-.63
13	1.26	55.84	56.59	1.34	79.96	80.24	.35
14	-9.64	51.81	54.42	5.04	113.04	121.09	7.12
15	14.19	62.86	60.13	-4.34	110.61	108.96	-1.49
16	-9.18	47.94	50.95	6.28	90.12	89.29	-.92
17	48.51	65.66	56.24	-14.35	97.20	96.52	-.70
18	-4.73	53.47	56.47	5.61	110.52	93.80	-15.13
19	.00	55.95	56.40	.80	80.37	86.16	7.20
20	-19.89	47.96	55.16	15.01	89.07	91.97	3.26

ID	preF66Trab CNT	stF66Trab CNT	cF66Trab CNT	preF66TOT A	postF66TOT A	pcF66TOT A	preF66Trab A
1	11.68	11.73	.43	167.68	162.56	-3.05	56.16
7	13.47	12.28	-8.83	140.32	140.48	.11	45.28
8	3.49	4.15	18.91	94.08	101.92	8.33	24.00
9	5.99	3.90	-34.89	83.84	80.32	-4.20	29.92
10	9.24	5.19	-43.83	133.92	116.16	-13.26	66.72
11	23.97	20.34	-15.14	167.04	162.56	-2.68	83.36
17	8.44	8.63	2.25	126.08	129.60	2.79	57.28
18	5.98	5.70	-4.68	106.08	106.24	.15	35.68
19	13.90	6.92	-50.22	135.04	125.12	-7.35	52.00
20	8.22	12.50	52.07	113.76	125.92	10.69	41.28
22	5.71	5.86	2.63	149.92	146.88	-2.03	57.60
23	7.62	15.60	104.72	125.92	150.40	19.44	51.20
25	3.15	3.33	5.71	101.44	105.12	3.63	20.00
26	8.29	11.03	33.05	100.96	110.40	9.35	44.64
28	2.87	3.22	12.20	94.08	93.28	-.85	23.04
29	12.18	11.19	-8.13	148.64	153.76	3.44	83.04
1	6.31	5.89	-6.66	139.84	139.20	-.46	66.88
2	9.08	8.15	-10.24	149.12	144.48	-3.11	53.12
3	10.47	11.07	5.73	115.84	112.32	-3.04	61.60
4	2.89	4.08	41.18	102.08	102.72	.63	24.48
6	15.05	16.82	11.76	127.04	128.48	1.13	69.12
7	7.96	6.93	-12.94	137.28	132.64	-3.38	53.76
8	6.83	7.13	4.39	114.72	116.96	1.95	53.92
9	11.50	10.50	-8.70	156.96	156.96	.00	75.04
11	4.22	4.58	8.53	85.76	85.44	-.37	25.12
12	7.80	7.64	-2.05	118.40	128.00	8.11	47.68
13	9.54	9.10	-4.61	145.12	143.04	-1.43	80.80
14	14.82	5.52	-62.75	140.48	127.68	-9.11	51.68
15	8.95	15.87	77.32	164.96	176.48	6.98	75.68
16	16.59	15.67	-5.55	124.64	123.52	-.90	56.00
17	9.36	9.63	2.88	132.80	146.08	10.00	55.20
18	8.05	4.14	-48.57	139.52	106.24	-23.85	46.88
19	6.96	7.27	4.45	109.60	117.44	7.15	45.76
20	9.92	6.20	-37.50	118.72	115.52	-2.70	46.88

ID	stF66Trab A	pcF66Trab A	preF66Cor CNT	66Cor CNT	f66Cor CNT	preF66Cor A	postF66Cor A	pcF66Cor A
1	53.28	-5.13	131.10	129.50	-1.22	111.52	109.28	-2.01
7	43.84	-3.18	109.33	108.89	-.40	95.04	96.64	1.68
8	28.16	17.33	83.84	87.03	3.80	70.08	73.76	5.25
9	23.84	-20.32	61.03	66.11	8.32	53.92	56.48	4.75
10	45.92	-31.18	75.45	81.21	7.63	67.20	70.24	4.52
11	73.28	-12.09	90.75	95.38	5.10	83.36	88.80	6.53
17	62.56	9.22	79.16	76.55	-3.30	68.80	67.04	-2.56
18	35.04	-1.79	79.37	79.83	.58	70.40	71.20	1.14
19	38.56	-25.85	93.47	99.14	6.07	83.04	86.56	4.24
20	54.08	31.01	83.00	79.32	-4.43	72.48	71.84	-.88
22	56.32	-2.22	106.91	105.67	-1.16	92.32	90.56	-1.91
23	85.28	66.56	84.26	70.28	-16.59	74.72	65.12	-12.85
25	20.96	4.80	98.62	100.76	2.17	81.44	84.16	3.34
26	52.80	18.28	63.55	62.72	-1.31	56.32	57.60	2.27
28	23.36	1.39	84.60	84.08	-.61	71.04	69.92	-1.58
29	85.28	2.70	74.37	77.88	4.72	64.48	68.16	5.71
1	65.76	-1.67	81.69	82.50	.99	72.96	73.44	.66
2	51.36	-3.31	104.61	103.70	-.87	96.00	93.12	-3.00
3	60.64	-1.56	61.70	57.36	-7.03	54.24	51.52	-5.01
4	26.08	6.54	93.65	92.26	-1.48	77.60	76.64	-1.24
6	84.00	21.53	59.83	43.38	-27.49	57.76	42.40	-26.59
7	52.00	-3.27	96.99	93.48	-3.62	83.52	80.64	-3.45
8	54.88	1.78	68.04	68.12	.12	60.80	62.08	2.11
9	74.88	-.21	93.31	93.50	.20	81.92	82.08	.20
11	25.92	3.18	70.52	69.94	-.82	60.64	59.52	-1.85
12	55.52	16.44	82.23	81.82	-.50	70.72	72.48	2.49
13	77.92	-3.56	70.42	71.13	1.01	64.32	65.12	1.24
14	27.84	-46.13	98.07	115.58	17.85	88.64	99.84	12.64
15	91.52	20.93	101.65	92.13	-9.37	89.28	83.68	-6.27
16	55.20	-1.43	73.40	73.62	.30	68.48	68.32	-.23
17	67.84	22.90	87.85	86.89	-1.09	77.60	78.24	.82
18	28.48	-39.25	102.47	89.65	-12.51	92.64	77.76	-16.06
19	48.64	6.29	73.41	78.89	7.46	63.84	68.80	7.77
20	40.64	-13.31	79.15	85.76	8.35	71.84	74.88	4.23

ID	preF66C Thk	stF66C Thk	pcF66C Thk	preF66Peri C	stF66Peri C	66Peri C	preHS	postHS	TKcal
1	3.08	3.08	.00	45.90	45.20	-1.53	1.006	1.017	2078
7	2.89	2.95	2.08	41.99	42.02	.07	1.020	1.006	1697
8	2.71	2.70	-.37	34.38	35.79	4.10	1.006	1.006	2172
9	2.08	2.30	10.58	32.46	31.77	-2.13	1.015	1.019	1702
10	1.92	2.26	17.71	41.02	38.21	-6.85	1.004	1.002	1213
11	2.13	2.35	10.33	45.82	45.20	-1.35	1.013	1.014	1501
17	2.07	1.96	-5.31	39.80	40.36	1.41	1.006	1.011	1204
18	2.44	2.48	1.64	36.51	36.54	.08	1.010	1.020	
19	2.49	2.81	12.85	41.19	39.65	-3.74	1.002	1.002	1509
20	2.39	2.18	-8.79	37.81	39.78	5.21	1.004	1.005	1847
22	2.63	2.60	-1.14	43.40	42.96	-1.01	1.006	1.002	1102
23	2.29	1.71	-25.33	39.78	43.47	9.28	1.009	1.003	2620
25	3.16	3.20	1.27	35.70	36.35	1.82	1.005	1.002	1925
26	1.90	1.83	-3.68	35.62	37.25	4.58	1.021	1.013	1701
28	2.76	2.72	-1.45	34.38	34.24	-.41	1.008	1.002	2175
29	1.70	1.78	4.71	43.22	43.96	1.71	1.007	1.003	1533
1	2.06	2.08	.97	41.92	41.82	-.24	1.015	1.011	1471
2	2.78	2.74	-1.44	43.29	42.61	-1.57	1.003	1.003	2117
3	1.64	1.58	-3.66	38.15	37.57	-1.52	1.008	1.025	756
4	2.91	2.84	-2.41	35.82	35.93	.31	1.012	1.002	1466
6	1.66	1.16	-30.12	39.96	40.18	.55	1.002	1.013	2515
7	2.47	2.43	-1.62	41.53	40.83	-1.69	1.005	1.002	2141
8	1.90	1.92	1.05	37.97	38.34	.97	1.004	1.024	7923
9	2.18	2.19	.46	44.41	44.41	.00	1.016	1.022	1893
11	2.40	2.34	-2.50	32.83	32.77	-.18	1.006	1.005	2745
12	2.24	2.18	-2.68	38.57	40.11	3.99	1.005	1.010	1516
13	1.73	1.77	2.31	42.70	42.40	-.70	1.014	1.022	3401
14	2.63	3.40	29.28	42.02	40.06	-4.66	1.008	1.024	2972
15	2.34	2.06	-11.97	45.53	47.09	3.43	1.006	1.007	1775
16	2.07	2.08	.48	39.58	39.40	-.45	1.003	1.008	2353
17	2.31	2.17	-6.06	40.85	42.85	4.90	1.004	1.002	2977
18	2.80	2.80	.00	41.87	36.54	-12.73	1.004	1.006	2576
19	2.09	2.18	4.31	37.11	38.42	3.53	1.013	1.025	1977
20	2.28	2.47	8.33	38.63	38.10	-1.37	1.029	1.016	2468

ID	Protein	Carb	Fat	VitD	alciumDD	Magn	BPAQ Pre	BPAQ CPre	AQ CPost	CBPAQ 2M	CBPAQ 4M
1	55.30	292.00	81.50	2.63	1135.64	564.98	3.85	7.69	6.56		
7	55.59	206.67	75.90	32.86	578.97	200.02	9.02	.25	.27		
8	69.06	285.03	87.15	3.02	622.78	186.37	12.68	13.62	.34		
9	124.60	191.83	52.83	1.31	342.82	190.90	1.12	.42	.24		
10	63.82	159.97	40.51	.29	699.24	111.94	68.67	3.17	4.22		
11	48.62	186.83	67.93	1.39	462.33	98.77	22.64	12.50	.27		
17	53.60	166.04	36.82	10.66	540.46	179.97	26.90	.00	.27		
18							71.91	6.38	.05		
19	78.90	127.26	73.12	.97	743.35	277.74	4.69	.72	.47		
20	51.89	248.48	74.15	1.40	988.70	109.88	21.07	.40	.23		
22	52.01	128.82	44.15	.25	483.38	91.68	28.75	9.41	3.34		
23	78.37	338.00	109.18	3.00	975.33	314.97	1.60	.37	.23		
25	75.52	217.96	82.11	.79	694.98	151.24	12.22	.35	.05		
26	102.10	136.72	82.59	4.94	1322.26	168.24	75.53	.72	.43		
28	107.89	209.40	102.16	2.42	631.28	334.92	1.62	1.44	.74		
29	56.35	216.69	55.50	.21	1015.36	186.89	1.36	.00	3.44		
1	60.43	180.68	59.39	1.48	588.03	216.39	69.05	8.41	2.92	.00	4.26
2	75.23	248.68	95.01	2.62	452.77	312.77	53.85	3.63	.48	2.44	4.00
3	33.36	86.17	33.20	.04	539.59	75.72	24.30	.00	.00	.00	.00
4	70.37	206.57	39.85	1.83	778.41	166.57	30.32	10.08	.64	.64	.64
6	89.41	296.80	113.72	6.98	1557.78	384.23	17.21	.00	.51	.40	2.04
7	88.88	263.05	86.89	5.57	1100.44	269.06	64.44	.88	1.00	.88	.56
8	197.39	991.18	368.06	16.94	5863.39	278.74	70.81	35.94	.00	6.83	.00
9	89.46	233.64	74.87	18.23	1311.30	750.08	34.50	.00	.52	.56	.64
11	193.93	311.64	75.66	19.19	2693.21	581.32	19.02	2.34	.36	4.59	4.10
12	69.74	222.74	40.46	.14	1083.63	362.52	1.50	.00	.00	.00	.00
13	55.28	577.77	111.51	11.33	649.97	268.03	8.43	.80	.48	.50	.50
14	118.86	379.37	111.85	11.99	2150.24	219.73	36.17	38.78	6.19	8.51	7.51
15	69.65	187.79	83.13	.00	524.28	210.30	7.05	.00	.52	.40	.56
16	86.08	207.84	139.48	.47	1016.22	672.02	6.51	.00	.56	.56	.88
17	102.56	298.01	133.03	5.70	1007.04	247.54	1.63	.64	.56	.00	.56
18	108.32	364.73	81.97	.12	542.98	217.75	2.84	.00	.48	.48	.48
19	83.00	257.47	71.85	2.00	1092.33	279.36	11.05	.00	.28	.00	19.07
20	106.69	315.25	92.27	12.93	1592.21	313.69	3.03	.72	.42	.40	2.80

ID	CBPAQ 6M	preTBMDT	postTBMDT	preRTHIPT	stRTHIPT	preRFNT	postRFNT	preRTCT	postRTCT	preLTHIPT
1		1.6	1.6	1.1	1.1	.4	.4	.6	.6	1.0
7		.4	.3	-.4	-.3	-.8	-.9	-.6	-.3	.1
8		.3	.3	-1.2	-1.2	-1.1	-1.2	-1.8	-1.8	-.7
9		-.9	-.9	-.7	-.8	-.6	-.8	-1.0	-1.1	-.8
10		-1.1	-1.1	-1.6	-1.7	-1.7	-1.8	-2.1	-2.2	-1.9
11		2.5	2.1	1.7	1.6	.5	.1	1.7	1.5	1.6
17		.7	.6	-.4	-.2	-.9	-.8	-.3	-.3	-.3
18		2.5	2.5	1.5	1.5	.6	.9	1.5	1.5	1.6
19		.7	.4	-.3	-.2	-.3	-.4	-1.1	-1.0	.0
20		-.1	.1	-1.0	-1.1	-1.3	-1.5	-1.1	-1.2	-.8
22		.6	.4	.9	.8	.3	.3	.4	.1	.4
23		-.7	-.5	-1.5	-1.6	-2.2	-2.3	-1.9	-2.0	-1.3
25		.8	.7	1.0	.9	.2	.0	.3	.2	.8
26		.1	.3	.0	.1	.0	-.1	-.3	-.2	.2
28		1.0	.8	-.5	-.4	-.7	-.5	-1.3	-1.3	-.2
29		.5	.3	-1.1	-1.0	-1.0	-1.1	-1.5	-1.3	-.8
1	4.26	1.0	.9	-.5	-.5	-1.1	-1.1	-1.1	-1.1	-.1
2	3.64	1.9	1.9	.6	.7	-.2	-.2	.3	.3	1.0
3	.00	-.5	-.6	-.2	-.2	-.1	-.5	-.9	-.8	-.2
4	.64	1.2	1.1	.8	.8	.4	.4	.4	.4	.6
6	.40	-.3	-.6	-.3	-.3	-.5	-.4	-.9	-1.0	-.8
7	.52	1.6	1.7	1.7	1.6	1.3	1.1	1.1	1.0	1.2
8	.00	.8	1.0	.1	.2	.3	-.1	-.3	-.2	-.2
9	.64	1.8	1.7	.0	.2	.0	.2	-.6	-.3	.6
11	.43	.4	.1	-.4	-.6	-.4	-.3	-2.0	-2.1	-.2
12	.00	.6	.4	-.2	-.2	-1.1	-1.1	-1.3	-.9	-.1
13	.72	-.5	-.4	-.3	-.3	-.3	-.3	-.9	-.8	-.2
14	4.54	1.0	1.0	.6	.7	.2	.5	-.3	-.1	.8
15	.00	1.8	2.0	1.3	1.4	.7	.4	.2	.5	.7
16	.88	1.4	1.9	1.1	1.1	.2	.2	.5	.6	.9
17	.56	.3	.3	-.6	-.6	-1.5	-1.1	-1.3	-1.3	-.6
18	.68	.5	.4	.7	.7	.0	-.3	-.1	-.1	.4
19	.10	.3	.4	.2	.3	-.4	-.3	-.2	.2	-.2
20	.39	-.2	-.3	-.2	-.4	-.9	-.8	-.9	-1.1	-.6

ID	stLTHIPT	preLFNT	postLFNT	preLTCT	postLTCT	preL14T	postL14T	preL24T	postL24T	preR33T	postR33T
1	1.0	.3	.2	.5	.5	1.5	1.2	1.6	1.5	-.4	-.6
7	.1	-.3	-.4	-.5	-.3	-.1	-.1	-.9	-.8	-.7	-.4
8	-.7	-.7	-.6	-.9	-1.1	.2	.1	.3	.2	-.2	.0
9	-.8	-.6	-.8	-1.1	-1.2	-.9	-.8	-.9	-.8	-1.3	-1.6
10	-2.0	-2.0	-2.1	-2.4	-2.5	-1.7	-1.6	-1.9	-1.8	.1	.2
11	1.4	.5	.2	1.3	.9	1.8	1.9	2.0	2.2	.2	.1
17	-.3	-.8	-.9	-.5	-.5	.7	.5	.6	.5	.0	.0
18	1.8	.7	1.0	1.8	1.9	2.6	2.7	2.5	2.7	.5	.2
19	.0	-.4	-.5	-.6	-.7	-.4	.0	-.5	-.2	.2	.0
20	-.9	-1.5	-1.5	-1.1	-1.2	.0	-.1	.0	-.1	-.7	-1.0
22	.4	-.2	.0	.0	-.2	.3	-.2	.3	-.2	.7	.4
23	-1.4	-2.2	-2.2	-1.9	-1.9	-1.4	-1.4	-1.3	-1.4	-.1	-.1
25	.8	.7	.5	.0	.0	.4	.5	.5	.5	.9	.1
26	.3	-.1	.1	-.1	.0	.6	.4	.7	.4	.4	.5
28	-.3	-.2	-.6	-1.3	-1.3	1.8	2.3	1.7	2.3	.7	.1
29	-1.0	-.8	-1.2	-1.4	-1.4	-.5	-.7	-.4	-.7	-.7	-.7
1	-.2	-1.0	-1.1	-.6	-.7	.3	.2	.4	.2	-1.8	-1.7
2	1.1	.1	.0	.8	.8	2.0	1.7	2.3	1.8	.0	-.2
3	-.2	-.2	-.3	-.7	-.6	.3	.4	.2	.3	-.7	-.3
4	.6	.1	.2	.2	.1	.1	.2	.1	.1	.3	.2
6	-.8	-.9	-.9	-1.3	-1.3	.0	.3	.2	.4	-1.2	-.8
7	1.3	.5	.6	.4	.4	2.0	1.8	2.0	1.6	.5	.7
8	-.1	.1	.0	-.5	-.4	.6	.9	.6	.9	-1.4	-1.1
9	.5	.3	.3	.2	-.1	1.2	1.2	1.2	1.2	-.1	-.2
11	-.2	-.4	-.3	-1.6	-1.4	-1.1	-1.3	-1.1	-1.2	-.6	-.5
12	-.1	-.8	-.8	-.6	-.6	-.4	-.3	-.5	-.4	.0	-.1
13	-.3	-.4	-.6	-.7	-.7	-.1	-.4	-.1	-.4	-.4	-.5
14	.8	.3	.5	-.1	.1	.2	.3	.4	.4	1.8	1.7
15	.9	-.3	.3	-.1	.1	1.5	1.2	1.5	1.0	.1	.2
16	.8	.2	.2	.5	.6	1.7	1.9	1.9	1.8	.0	-.3
17	-.8	-.9	-.9	-1.2	-1.4	-.2	.0	-.1	.0	-.3	-.7
18	.5	-.5	-.6	-.3	.0	.4	.5	.4	.5	-.4	-.1
19	.0	-.9	-.7	-.3	-.2	.4	.1	.4	.3	-1.2	-.7
20	-.6	-.8	-.8	-1.3	-1.4	-1.3	-1.1	-1.0	-.8	.3	.4