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Changes in Bone Mineral Density in Middle-Age Women According to Physical Activity Volume, Intensity, and Cardiorespiratory Fitness: A Six-Year Prospective Study

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CHANGES IN BONE MINERAL DENSITY IN MIDDLE-AGE WOMEN ACCORDING TO PHYSICAL ACTIVITY VOLUME, INTENSITY, AND CARDIORESPIRATORY FITNESS: A 6-YEAR PROSPECTIVE STUDY

by

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GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

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This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the dissertation of Neil R. Nokes in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

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This study was conducted to determine if physical activity and cardiorespiratory fitness (CRF) at baseline influence the likelihood of gaining bone mineral density (BMD) at the hip and lumbar spine over 6 years. Another aim was to ascertain the effect of several potential confounding factors.

In a prospective study of 244 women (baseline age range 35-45 years), physical activity volume (PAv) and intensity (PAi) were measured using accelerometers at baseline. CRF indexed by VO2max was estimated using a graded, maximal treadmill test at baseline. BMD was measured using DEXA. Risk ratios were used to show the likelihood of BMD gains (> 75th percentile) between different levels of PAv, PAi, or CRF at baseline.

Mean hip BMD change was -0.015 + 0.045 g/cm2. Women with high PAv were 2.50 times (95% CI: 1.19-5.24), and women with moderate PAv were 2.20 times (95% CI: 1.08-4.45), more likely to experience significant hip BMD gains than women with low PAv. Adjusting for potential confounders had little effect on the results. Baseline PAi and CRF were not related to changes in hip BMD. None of the relationships between PAv, PAi, and CRF, and changes in spine BMD, was statistically significant.

Middle-aged women with moderate or high levels of PAv are more likely to experience significant gains in hip BMD over time compared to those with low levels of PAv.

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Changes in Bone Mineral Density in Middle-Age Women By Physical Activity Volume, Intensity, and Cardiorespiratory Fitness: A 6-year Prospective Study

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ABSTRACT

Background: This study was conducted to determine if physical activity and cardiorespiratory fitness (CRF) at baseline influence the likelihood of gaining bone mineral density (BMD) at the hip and lumbar spine over 6 years. Another aim was to ascertain the effect of several potential confounding factors. **Methods:** In a prospective study of 244 women (baseline age range 35-45 years), physical activity volume (PAv) and intensity (PAi) were measured using accelerometers at baseline. CRF, indexed by VO_2max was estimated using a graded, maximal treadmill test at baseline. BMD was measured using DEXA. Risk ratios were used to show the likelihood of BMD gains (> 75th percentile) between different levels of PAv, PAi, or CRF at baseline. **Results:** Mean hip BMD change was -0.015 $\pm$ 0.045 g/cm^2. Women with high PAv were 2.50 times (95% CI: 1.19-5.24), and women with moderate PAv were 2.20 times (95% CI: 1.08-4.45), more likely to experience significant hip BMD gains than women with low PAv. Adjusting for potential confounders had little effect on the results. Baseline PAi and CRF were not related to changes in hip BMD. None of the relationships between PAv, PAi, and CRF, and changes in spine BMD, was statistically significant. **Conclusions:** Middle-aged women with moderate or high levels of PAv are more likely to experience significant gains in hip BMD over time compared to those with low levels of PAv.

INTRODUCTION

Age-related deterioration of bone micro-architecture and low bone mass may be partly due to menopause or ovarian failure, bone remodeling dysfunction, malnutrition, liver and kidney diseases, endocrine disorders, low BMI, and physical inactivity.[1-5] Disuse and inactivity lead to bone loss, whereas weight-bearing physical activity (PA) may maintain or improve bone mineral density (BMD) by transmitting mechanical stresses to bone both directly and indirectly.[2, 6, 7] According to Wolff's law, osteoclasts and osteoblasts directly optimize bone mass, geometry, and material properties to withstand habitual loading.[7, 8] Indirect mechanisms occur through bone-muscle interactions described by the theory of mechonstat.[7] Overtime, greater mechanical loads and muscle-bone interactions cause deposits of bone mineral matrix to strengthen the bone, which increases its BMD.[2]

Bone's response to load is highly dependent on the magnitude, rate, distribution, and cycles of strain.[7, 8] Different volumes and intensities of PA translate into different magnitudes, rates, distribution and cycles of loads, which appear to cause different levels of strain on bone, initiating different osteogenic responses and different densities.[2, 7, 8] Hence, in theory, bone mineral density should be related to an individual's habitual PA pattern.

Physical activity through the fourth decade of life in women is an important determinant of BMD.[2, 5, 6, 9-12] Increasing PA to improve BMD may represent a feasible strategy for primary prevention of osteoporosis.[5, 6, 13-17] However, there are few studies of the effects of physical activity on BMD of the hip and spine in middle-age women over

significant lengths of time.[18-21] Furthermore, experimental studies on this relationship in middle-age women have inconsistent findings, with a few studies observing gains in BMD, some showing maintenance of BMD, and others revealing loss of BMD.[7, 20, 22-27]

Prospective cohort studies reveal clarifying information on the influence of PA on BMD over several years.[2, 13, 28] This type of study could be enlightening regarding the theory that sustained high levels of PA yield greater BMD levels which serve as a reservoir of bone mass and a protection from osteoporosis.[2, 5, 29] Prospective studies may support the hypothesis that PA protects against osteoporosis through increased BMD,[5, 17, 30, 31] "but the quality of the evidence supporting this notion is weak."[19]

The quality of evidence in prospective cohort studies is weak possibly because PA measurements are typically based on questionnaires and are rarely measured objectively.[7,27, 28] Questionnaires are subject to recall bias, are unable to account for daily PA, and are often inflated accounts of PA.[32-34] Furthermore, previous studies have typically assessed PA by asking subjects to report the number of hours per week they exercise, which does not quantify physiologic strain frequencies.[7] The adaptive response of bone is sensitive to strain number, strain duration, strain intensity and impact, but more so the latter two.[7] Thus, to evaluate the influence PA has on BMD, volume and intensity of PA should be considered.[7, 30, 35, 36]

Accelerometers can objectively measure volume of PA over a given time period (e.g., 7 days).[17, 37, 38] Furthermore, intensity of PA can be deduced by dividing volume of PA by a specific time or bout (e.g., 10 minutes). Thus, variations in activity counts within

a specific time period reflect differences in PA intensity (PAi).[39-41] Thus, volume and intensity of PA can be measured objectively.

Physical activity is only one aspect of lifestyle that affects BMD.[7, 42] Cardiorespiratory fitness (CRF), indexed by VO_2max, is a function of PA volume (PAv), PAi, and percent body fat, which all correlate with BMD.[42-45] However, the relationship between VO_2max and BMD is questioned in the literature.[46] Older studies have reported a significant relationship between CRF and BMD,[42, 47] while more recent research has not observed a significant relationship.[46]

The theory supporting the CRF—BMD relationship is that CRF is a surrogate of training status or habitual intense PA, various lifestyle factors, and mechanical load placed on the skeleton.[42] Thus, the two should be positively correlated. Furthermore, an individual's CRF should reveal the nature of an adult's long-term PA, because CRF is significantly related to habitual PAi.[42, 43]

Bone mineral density is associated with habitual PA and lifestyle factors of young women, but this relationship is not well supported in middle-age females.[2, 6, 9-11, 18-21] The theory behind this relationship is plausible and prospective cohort studies are a good source for evidence of the PA—CRF—BMD relationships.[2, 13, 28] However, PA is rarely measured objectively using accelerometers.[7, 27, 28] Therefore, a prospective cohort design studying the relationship between PA using accelerometers, and BMD measured by DEXA, would be a worthwhile addition to the body of literature on bone mineral density in midlife women. Furthermore, clarification of the relationship between CRF, indexed by VO_2max, and BMD is warranted because of the inconsistencies found in the literature.

METHODS

Design

This study used a prospective cohort design measuring physical activity volume (PAv), physical activity intensity (PAi), and cardiorespiratory fitness, indexed by VO_2max. Bone mineral density (BMD) at the hip and spine and potential confounding variables, including age, baseline hip or spine BMD, baseline body mass, baseline CRF or PAv, mass change, time in the study, menopause status, mean calcium intake, mean vitamin D intake, perceived changes in fitness, maternal history of osteoporosis, and prescription drug use for osteoporosis, were also measured. The study was approved by the university Institutional Review Board before data were collected at baseline and again at follow-up.

Participants

A power analysis was conducted using the PASS 6.0 statistical software to determine the number of participants needed when analyzing a 3 x 2 contingency table using chi-square to detect an effect size of 0.25 with power at 0.80 and alpha at the 0.05 level. Results showed that 155 subjects would be sufficient.

At baseline, a total of 268 women were recruited using newspaper advertisements and flyers distributed throughout more than 20 cities in the Mountain West. At baseline, the sample was delimited to nonsmokers, ages 35-45 years, and apparently healthy women based on a physical activity readiness questionnaire. Additionally, the sample was approximately 90% white, 80% married, and 37% reported some college education.

On average, follow-up occurred six years after baseline (mean ± SD) (2193 ± 720 days). Because loss to follow-up was a concern, participants were offered $100 cash to return for the follow-up assessments. A total of 244 participants of the original cohort returned for the follow-up, a 9.0% attrition rate. Women who failed to return for the follow-up assessments did not differ significantly from those who completed both phases of the study regarding age (P = 0.85), baseline hip BMD (P = 0.85), baseline spine BMD (P = 0.24), baseline weight (P = 0.96), CRF (P = 0.15), PA (P = 0.67), and perceived fitness (P = 0.34).

Procedures

At baseline, participants came to the lab for two separate appointments one week apart. For the first appointment, women came to the lab fasting for at least three hours. After an explanation of the study, participants read and signed the informed consent and were then asked to eliminate any body waste and change into a snug-fitting, standardized, one-piece swimsuit. Participants were weighed to the nearest 0.05 kg using a calibrated electronic scale. After weighing, participants had their hip and spine scanned on the bone densitometer.

After the bone scans were completed, participants changed into exercise clothing and performed a graded maximal treadmill test (GXT) according to the modified Arizona State University protocol.[48] At the end of the first appointment, participants received an accelerometer and instructions regarding how to wear the accelerometer. Also, participants were issued a questionnaire and the Block food frequency questionnaire to be filled out during the upcoming week.

One week later, participants came back to the lab and returned the accelerometer and the questionnaires. The questionnaires were reviewed for errors and participants were contacted to correct mistakes. At follow-up, the same methods were used, except there was no physical activity monitoring or GXT assessment.

Instrumentation and Measurements

At baseline, age, physical activity volume (PAv), physical activity intensity (PAi), CRF, bone mineral density at the hip and spine, mass weight, intake of calcium and vitamin D, menopause status, and perceived fitness were measured. At follow-up approximately six years later, the same variables were measured, except PAv, PAi, and CRF. Prescription drug use to increase BMD and maternal history of osteoporosis were measured as part of the follow-up protocol only.

Physical Activity

At baseline, participants wore Computer Science Application, Inc (CSA, now Actigraph, Pensacola, FL) model 7164 accelerometers to measure physical activity objectively. Computer Science Application accelerometers have been shown to be both valid and reliable in estimating physical activity in the field.[49, 50] In a study comparing four popular activity monitors, the CSA accelerometer was the most accurate during moderate intensity physical activities in field and laboratory settings.[49] Furthermore, these accelerometers were strongly correlated to oxygen consumption measures obtained from portable metabolic systems.[49, 50] Computer Science Application activity counts have been reported to be highly correlated to steady-state oxygen consumption and significantly correlated with relative VO_2, heart rate, and treadmill speed.[50, 51]

Participants were instructed regarding how to wear the monitor and they were encouraged to wear it at all times, except when engaging in water activities. Participants carried the accelerometer in a small pouch on a nylon belt at the height of the umbilicus, over the left hip, and along the outer seam of a pair of pants. Participants were required to wear the accelerometers for seven consecutive days to provide extended evaluation of habitual physical activity. If participants failed to wear the activity monitor for more than two hours on any day or four hours or more during the week, they were required to wear the accelerometer for another seven consecutive days. The accelerometers recorded movement continuously and, in a week's time, most individuals had activity count totals in the millions. The PAv variable was indexed using the sum of all the activity counts over the 7-day period.

Variations in accelerometer activity counts within a specific time period (e.g., 10 minutes) reflect differences in PAi.[39-41] Published reference criteria for three PAi categories reflecting low, moderate, and vigorous physical activity intensities have been established.[39-41] The criteria define the low PAi category as sedentary living up to walking slowly (< 3mph) with activity counts ranging from 0 to 29,000 per 10-minute interval.[40] The moderate PAi category has been defined as walking slowly to briskly (3 to 4 mph) and includes the range of 30,000 to 50,000 counts per 10-minutes.[40] Finally, the vigorous PAi category includes movement more intense than brisk walking (> 4 mph) with activity counts greater than 50,000 per 10-minute interval.[40] Because the CDC and ACSM guidelines indicate that intermittent bouts of physical activity, as short as 10 minutes, totaling 30 minutes or more on a given day, provide beneficial health and fitness

effects,[52, 53] activity counts were divided into 10-minute intervals as minimum intermittent bouts to be consistent with the Centers for Disease Control and American College of Sports Medicine guidelines. This resulted in 1008 intervals for each participant over the seven-day testing period.

Given the 2007 American Heart Association and American College of Sports Medicine recommendation that adults should participate in moderately intense cardio at least 30 minutes per day, five days per week, (i.e., 150 minutes), in the present study, participants were required to accumulate at least 150 minutes (i.e., at least 15 ten-minute bouts) within a specific intensity range (Low, Moderate, or Vigorous) over the seven days to be classified in that intensity category.[53] For example, in order to be included in the Vigorous PAi category, participants had to have 50,000 counts or more in at least 15 intervals (i.e., 150 minutes) during the seven days. To be in the Moderate PAi category, participants had to have at least 150 minutes of activity counts of 30,000 or more and fail to fit into the Vigorous PAi category. For participants to be in the Low PAi category, they had to accumulate fewer than 15 intervals with intensities greater than 30,000 counts. If participants had 14 Vigorous intensity intervals and 7 Moderate intensity intervals, then they were placed in the Moderate PAi category, because they had at least 15 intervals with 30,000 counts or more and they did not have at least 15 intervals with 50,000 counts or more.

Cardiorespiratory Fitness

To ensure the safety of participants, the GXT was performed following guidelines from the American College of Sports Medicine (ACSM).[54] Participants performed the GXT following the modified Arizona State University (ASU) protocol for the estimation of VO_2max.[48] George[48] reported that this protocol has stable results across subgroups with high *r* values (≥ 0.95) and low SEE (< 2.0) generated by a double, cross validation procedure.[48] He also reported an intraclass test-retest reliability of the protocol to be 0.99.[48]

Following the modified ASU protocol, participants walked on a motor-driven Quinton Model 65 treadmill (Quinton, Seattle, WA) at a 5% grade at a self-selected, brisk pace during the first 3-minute stage of the GXT. At the beginning of the second 3-minute stage, participants selected a comfortable jogging pace after the grade was decreased to zero degrees. Following the second 3-minute stage, the treadmill grade was increased by 1.5% every minute until the participant could not continue because of volitional fatigue.

A Polar electronic monitoring system (Polar Inc., Westbury, NY) measured the participants' heart rates. Participants reported their perceived exertion by selecting a rating of perceived exertion (RPE) from a scale of 6 to 20 based on the Borg scale.[48] The examiner recorded the heart rates and RPE after the two 3-minute stages and at the end of each subsequent minute.

VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) was estimated based on the grade and speed of the treadmill during the last stage completed by the subject, in other words, the last full minute.[48] The GXT was considered maximal when subjects could not continue the

treadmill test, RPE reached 19 or 20, and heart rate was within 15 beats of the predicted maximal heart rate (220 - age). Participants (n = 7) were retested approximately one week later if they failed to achieve a maximal test.

Bone Mineral Density

Bone mineral density of the hip and lumbar spine (i.e., L1-L4) was measured using dual energy x-ray absorptiometry (DEXA), more specifically a Hologic QDR 4500 W (Hologic, Waltham, MA) bone densitometer. DEXA has been reported to be a reliable and valid procedure for measuring bone mass and density as a surrogate of fracture risk.[8, 18, 55] The 6-year follow-up employed the same procedure using the same Hologic QDR 4500 W bone densitometer to measure BMD at the hip and spine for the outcome variables.

On each day of testing, the technician calibrated the DEXA before any scans were performed. Participants were positioned on the DEXA facilitated by a laser light cross-hairs emitted from the scan arm to improve accuracy of the scan. Scans were analyzed using the QDR 11.2 software (Hologic, Waltham, MA) to determine participants' BMD.

For the hip scan, the laser cross-hairs were positioned so they bisected the left thigh at the level of the pubic bone. The technician internally rotated the participants' left leg, slightly abducted the leg, and strapped it to a positioning aide. This position minimized the view of the lesser trochanter and aligned the femoral shaft vertically on the scan image.

For the spine scan, a rectangular foam box was positioned under the participant's lower legs to flatten the lordosis of the spine. The cross-hairs of the laser were aligned to

bisect the body vertically, one inch below the highest level of the iliac crest. This positioning allowed for scanning L1-L4 vertebral bodies and increased accuracy of the spine scan.

Questionnaire

A questionnaire was administered to assess a variety of potential confounding variables. Specifically, questions were asked regarding use of prescription drugs to enhance bone health, menopause status, maternal history of osteoporosis, and perceived fitness at baseline and follow-up.

Many women in the U.S. take medication prescribed by their physician to slow the loss of bone and, when possible, increase BMD.[3, 56, 57] Use of bone medication was assessed in the present study by asking participants if they had used bone drugs prescribed by a physician to increase BMD in the last 10 years and how long the drugs had been taken.

Research shows that menopause status plays a significant role in the bone health of women.[58] Menopause status was evaluated using a series of six questions which ascertained symptoms of menopause, characteristics and duration of the menstrual cycle, time since the last menstrual cycle, and related factors. Using the questionnaire results, participants were labeled as premenopausal (n = 126), perimenopausal (n = 38), postmenopausal (n = 49), or had a hysterectomy (n = 31). A sample of 198 women was used to validate the menopause questionnaire results using a blood test measuring follicle stimulating hormone (FSH), an objective measure of ovarian function and ovulation status. Results of the menopause questions and blood test were highly correlated (F =

52.3, $R^2 = 0.45$, $P < 0.0001$), indicating a substantial degree of concurrent validity. Specifically, women who were labeled post-menopausal, perimenopausal, hysterectomy, or premenopausal, according to the questionnaire, had FSH levels (mIU/mL) of 67.1, 41.2, 32.9, and 9.2. Each of the groups differed significantly from each other except the perimenopausal and hysterectomy groups.

Osteoporosis has a genetic component.[1, 18, 59] Women with mothers who developed osteoporosis are more likely to develop osteoporosis themselves.[1, 18, 59] Hence, each participant was asked to report if her biological mother had been diagnosed with osteoporosis by a physician before age 65. Participants who did not know the osteoporosis status of their mother could indicate that they did not know.

Perceived fitness was ascertained by a question administered at baseline and again at follow-up asking participants to report how difficult it would be to jog two-miles without stopping. Participants were given seven possible responses, extremely difficult, very difficult, difficult, in-between, easy, very easy, and extremely easy. If the same response was not given at baseline and follow-up, then participants were determined to have changed their perceived fitness level. If women recorded a response at follow-up reflecting greater difficulty than at baseline, they were categorized as “Losing Fitness” (n = 56; 23%), and if a more favorable response was recorded at follow-up than at baseline, women were classified as “Gaining Fitness” (n = 90; 37%). If the same response was given at baseline and follow-up, then women were categorized as having “No Change” (n = 98; 40%).

Body Mass

Body mass at baseline was measured to the nearest 0.05 kg using an electronic scale with a computer interface (Life Measurement Instrument, Concord, CA). The scale was calibrated each day using known weights. Body mass was also measured again at follow-up using the same scale. A test-retest check of reliability using all participants was conducted requiring participants to be measured twice after complete repositioning. The intraclass correlation was extremely high ($R^2 = 0.999$, $P < 0.0001$) showing excellent reliability.

Average Intakes of Calcium and Vitamin D

The Block Food Frequency Questionnaire (FFQ) assesses the portion size and frequency of consumption of over 100 different foods and drinks and is considered a valid and reliable measure of micro- and macro-nutrients and food intake.[60-63] The Block FFQ measures separately intakes of calcium and vitamin D in foods and beverages, and supplements.[4, 18, 64] Numerous studies indicate that dietary and supplemental calcium and vitamin D influence bone mineral density and health in women.[65]

In the present study, calcium and vitamin D from foods and beverages, and also supplements, were combined, providing two measures, one of total calcium intake and one of total vitamin D consumption. Total intake of calcium and vitamin D at baseline and at follow-up were averaged and used as potential confounding factors.

Statistical Analysis

Descriptive data, including frequencies, means, and standard deviations, were generated for each variable. Changes in BMD and body mass were calculated by

subtracting baseline values from follow-up values taken approximately six years later. Changes from baseline to follow-up were examined for statistical significance using the paired t-test.

The principal outcome variable of the present study was BMD change, specifically BMD change of the total hip and lumbar spine. Based on BMD change scores, participants were divided into quartiles and the middle-two quartiles were collapsed forming three categories, BMD loss ($< 25^{th}$ percentile), minimal change of BMD ($25^{th} – 75^{th}$ percentile), and BMD gain ($> 75^{th}$ percentile). For the measures of physical activity volume (PAv) and cardiorespiratory fitness (CRF), participants were also divided into quartiles and the middle-two quartiles were collapsed forming three categories for each variable (Low, Moderate, and High). Lastly, participants were also divided into three groups based on their physical activity intensity (PAi) levels, as discussed previously.

Mantel-Haenszel chi-square analyses were conducted to determine the linear associations between PAv, PAi, and CRF, treated as ordinal categorical variables, and the outcome variables, BMD changes of the hip and spine, also treated as ordinal categorical variables. Relative risk was calculated using the PROC GENMODE command in SAS by comparing incidence rates over the six-year study. For the relative risk calculations, statistical significance was determined using 95% confidence intervals. Specifically, probability of BMD gains at the hip and the spine were determined by comparing the incidence rate of BMD gains among women with high and/or moderate levels of PAv to those with low levels of PAv. Similarly, likelihood of BMD gains was determined by

comparing the incidence rates of women with vigorous and/or moderate PAi levels to those with low PAi levels. Similar comparisons were made across the CRF categories as well.

The relative risk calculations were adjusted for the effects of several potential confounding variables, including age, baseline hip or spine BMD, baseline body mass, baseline CRF or PAv, mass change, time in the study, menopause status, mean calcium intake, mean vitamin D intake, and perceived changes in fitness, treated individually and in combination, using a modified Poisson regression approach with robust error variance, as explained by Zou.[66]

Only 22 participants reported use of a prescription drug to improve bone health, and these women did not differ significantly from their counterparts regarding BMD change across the study, so bone-drug use was not included as a covariate. Likewise, maternal history of osteoporosis was unrelated to changes in BMD, so it was not included in the final analysis as a possible confounding variable. All statistical analyses were computed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Between the baseline and follow-up assessments, on average, there were 2193 $\pm$ 720 days (i.e., six years). Over the six years, 59% of the participants lost BMD at the hip. As shown in Table 1, baseline average hip BMD was 0.934 $\pm$ 0.114 g/cm^2 and follow-up mean hip BMD was 0.919 $\pm$ 0.117 g/cm^2. Mean hip BMD change was -0.015 $\pm$ 0.045 g/cm^2, a significant decrease over time. Of the three primary predictors, PAv, PAi, and

CRF, only PAv was a significant predictor of BMD gains at the hip over time. Table 1 provides descriptive information for the key variables at baseline and follow-up.

Baseline average spine BMD was 1.016$\pm$ 0.120 g/cm^2 and mean follow-up spine BMD was 1.005$\pm$ 0.133 g/cm^2. Mean spine BMD change was -0.010 $\pm$ 0.054 g/cm^2, reflecting a significant decrease over time. Of the three primary predictors, none predicted significantly BMD gains at the spine over time.

Baseline Physical Activity Volume and Changes in Hip BMD

At baseline, mean total activity counts per week (PAv) was 2.67 $\pm$ 0.93 million, which is very similar to other published weekly averages.[39-41] As shown in Table 2, women with higher volumes of PA were significantly more likely to gain hip BMD from baseline to follow-up than their counterparts (χ^2_{mh} = 6.1, P = 0.01). Specifically, 32.3% of women in the High PAv category experienced BMD gains and were in the highest quartile (> 75th percentile) of BMD change over the six years, whereas 28.3% of women with Moderate PAv and 12.9% of women with Low PAv were in the highest quartile of BMD change.

As shown in Table 3, women in the Moderate-High PAv category (i.e., Moderate and High groups combined) had 2.30 times (95% CI: 1.16-4.56) greater likelihood of significant hip BMD gains (> 75th percentile of BMD change), than women in the Low PAv group. After adjusting for differences in the potential confounding factors, including age, baseline hip BMD, baseline body mass, baseline CRF, mass change, time in the study, menopause status, mean calcium intake, mean vitamin D intake, and perceived

changes in fitness, likelihood of experiencing significant gains in hip BMD (> 75th percentile of BMD change) changed little (Table 3).

Adjusting for differences in baseline CRF levels had the greatest influence on likelihood of significant hip BMD gains, strengthening the relationship by 18%. Adjusting for differences in menopause status had the most negative effect on the relationship (-10%), but the relationship remained significant (P = 0.03). After controlling for all of the potential confounding factors simultaneously (Table 3), women with Moderate to High PAv levels were 2.45 times more likely to have significant hip BMD gains (> 75th percentile of BMD change) across the study compared to Low PAv women (P = 0.01).

When women in the High PAv category were compared directly to women in the Low PAv group, likelihood of significant gains in hip BMD (> 75th percentile of BMD change) was greater (Table 3). Those in the High PAv group were 2.50 times (95% CI: 1.19-5.24) more likely to experience significant BMD gains in the hip than women in the Low PAv group. After adjusting for all of the covariates simultaneously, likelihood of experiencing significant hip BMD gains was 4.08 times (95% CI: 1:43-11.60) greater for women with High PAv compared to those with Low PAv.

As shown in Table 3, women in the Moderate PAv group were 2.20 times (95% CI: 1.08-4.45) more likely to experience significant BMD gains in the hip (> 75th percentile of BMD change) than women in the Low PAv group. The Moderate and High PAv groups did not differ significantly regarding likelihood of gaining hip BMD across time

(P = 0.58), without and with control of the potential confounding variables. Table 4 reflects the relationship between PAv and each of the covariates.

Baseline Physical Activity Intensity and Changes in Hip BMD

There were three physical activity intensity (PAi) categories, Low, Moderate, and Vigorous. At baseline, the Low PAi category included 200 women, whereas the Moderate category had 27 and the Vigorous group included 17 participants. As shown in Table 2, women in the Low, Moderate, and Vigorous PAi categories did not differ in the percentage experiencing significant gains in hip BMD (> 75th percentile of BMD change) from baseline to follow-up (χ^2_{mh} = 0.1, P = 0.70). Specifically, 17.7% of women in the Vigorous PAi category experienced favorable BMD changes that were in the highest quartile, whereas 29.6% of women with Moderate PAi and 25.5% of women with Low PAi were in the highest quartile of BMD change. Likewise, there were no statistically significant relative risk comparisons using the PAi measure. In short, women in the Moderate and Vigorous PAi categories (i.e., Moderate and Vigorous PAi combined) did not differ in their likelihood of experiencing significant gains in hip BMD compared to women in the Low PAi category (RR = 1.02; 95% CI: 0.58-1.79). Similarly, women in the Moderate PAi category did not differ significantly from those in the Low PAi group (RR = 0.86; 95% CI: 0.46-1.61), whereas the comparison between those in the Vigorous and Low PAi categories could not be computed because of insufficient participants to cover all of the cells of the model (see Table 1 in the Addendum). Table 5 reflects the relationship between PAi and each of the covariates.

Baseline Cardiorespiratory Fitness and Changes in Hip BMD

At baseline, the average VO_2max score across all subjects was 36.9 $\pm$ 7.4 $mL \cdot kg^{-1} \cdot min^{-1}$, which is similar to the 50th percentile of women in their fourth decade according to norms of the Aerobic Center Longitudinal Study (i.e., 34.6 $mL \cdot kg^{-1} \cdot min^{-1}$).[67] As shown in Table 2, women with higher levels of CRF did not differ from those with lower levels of CRF regarding the percentage that experienced significant gains in hip BMD (> 75th percentile) from baseline to follow-up (χ^2_{mh} = 1.5, P = 0.23). Specifically, 18.5% of women in the High CRF category experienced gains that were in the highest quartile of BMD change, whereas 28.1% of women with Moderate CRF and 27.7% of those with Low CRF levels were in the highest quartile of BMD change. Similarly, none of the measures of relative risk were statistically significant.

As shown in Table 2 of the addendum, women in the High CRF category had 0.67 times (95% CI: 0.35-1.27) the likelihood of significant hip BMD gains (> 75th percentile of BMD change) compared to women in the Low CRF group. Adjusting for differences in the potential confounding factors, individually and in combination, had no meaningful influence on the results. Table 6 reflects the relationship between CRF and each of the covariates, whereas Table 7 displays the relationship between changes in hip BMD and each of the covariates.

Spine Bone Mineral Density

None of the relationships between baseline PAv and changes in spine BMD, baseline PAi and changes in spine BMD, nor baseline CRF and changes in spine BMD was statistically significant. Controlling for the potential confounding factors,

individually and combined, had no significant influence on the results. Tables 3, 4, and 5 in the addendum show the specific findings regarding PAv, PAi, CRF, and changes in spine BMD.

DISCUSSION

The present study used a prospective cohort design spanning 6 years with no intervention or treatment. Given these circumstances, left on their own, 41% of the women gained BMD in the hip over the investigation and 59% lost BMD. In short, changes in BMD varied significantly from baseline to follow-up, and volume of physical activity (PAv) was important in predicting BMD changes across the investigation.

Evidently, high levels of PAv in middle-aged women increase the likelihood of significant BMD gains in the hip by 150%, and moderate levels of PAv increase the probability of significant BMD gains by 120%, compared to low levels of PAv, over time. Moreover, the association between PAv and changes in hip BMD was robust. Although several potential confounding factors were controlled statistically, only two covariates, menopause status and baseline CRF, had any meaningful effect on the results. Adjusting for differences in menopause status weakened the relationship by 10%, but the relationship remained significant ($P = 0.03$). Post-hoc analyses showed that menopause status was significantly related to BMD changes in the hip ($F = 24.7$, $P < 0.0001$), with postmenopausal women showing significantly greater mean BMD losses than women who were premenopausal, perimenopausal, and those with hysterectomies. The relationship between menopause status and changes in hip BMD remained highly significant after controlling for potential confounding factors, such as age, baseline body

mass, and changes in body mass. Menopause status was also related significantly to baseline PAv ($F = 2.8$, $P = 0.04$), however, the association was nullified after adjusting for differences in age.

Controlling statistically for differences in baseline CRF levels positively influenced the likelihood of significant hip BMD gains, strengthening the relationship by 18% ($P = 0.01$). Post-hoc analyses showed that baseline CRF levels were not related to changes in hip BMD over the six years ($F = 0.2$, $P = 0.82$). However, baseline CRF levels were associated significantly with baseline PAv levels, accounting for 22% of the variance in PAv ($F = 33.9$, $P < 0.0001$). Apparently, it does not matter whether women begin with high or low levels of CRF, those who participate in moderate to high levels of physical activity over time are much more likely to experience significant gains in hip BMD compared to women who are sedentary.

Because adjusting for differences in age, time in the study, baseline hip BMD, baseline body mass, changes in body mass, average calcium intake, average vitamin D consumption, and changes in perceived fitness had no effect on the PAv and BMD gain relationship, it appears that women with high and moderate levels of these traits are just as likely to experience significant gains in hip BMD as women with low levels. In short, these factors seem to have little influence on the PAv—BMD gain association.

The present study is in agreement with a cross-sectional investigation by Uusi-Rasi et al.[16] who studied PA and hip BMD. These researchers concluded that BMD of the femoral neck was 5% higher in women with high PA compared to those with low PA.[16] High PA was related to “larger and mechanically more competent bones in the femoral

and radial shafts with the association being stronger with increasing age."[16] Results from the study by Uusi-Rasi et al.[16] suggest that moderate PA can result in "considerable long-term improvement in the mechanical competence of the skeleton,"[16] which may be an over-statement given the cross-sectional design of this study.

The present study supports the hypothesis that moderate and high volumes of physical activity increase the likelihood of BMD gains, as well.[68] For example, using DEXA, Goto et al. examined whether BMD of the proximal femur was maintained in pre-, peri-, and postmenopausal women by regular exercise.[68] A total of 26 Japanese women (mean age 47.8 years) was followed 4-5 years. Of the 26 women, 22 were athletes and had participated regularly in volleyball or jogging clubs and had engaged in the same exercise for more than 5 years at the initial BMD measurement. In the proximal femur of the athletes, BMD increased in the premenopause group, but decreased in the perimenopause group.[68] In the premenopause athletes, BMD of the proximal femur increased. However, in the proximal femur, the four non-athletic women showed a 0.31% decrease, a significant difference compared with the athletes ($P < 0.05$). Goto et al. suggest that women can achieve gains in bone mass in the proximal femur before menopause by regular PA.[68] However, continued PA in the perimenopausal women of this cohort was not able to prevent bone loss.[68]

Park and colleagues[69] evaluated associations between bone health and the quantity and quality of habitual PA in a cohort of 76 men and 96 women (65-83 yrs). A specially adapted accelerometer measured the number of steps taken and intensity of physical activity throughout each 24-hour period for 1 year. At the end of the year, a quantitative

ultrasonic technique assessed each participant's osteosonic index (OSI). Results showed that in both sexes the OSI score increased with increasing daily PA, up to the observed maximum values of approximately 14,000 steps/day and 50 min/day at an intensity >3 METs.[69] Multivariate-adjusted logistic regression analyses indicated that men and especially women who engaged in <6,800 steps/day and <16 min/day of moderate-intensity PA were, respectively, 4.9-8.4 and 2.2-3.5 times more likely to sustain fractures than those participating in >8,200 steps/day and >25 min/day of activity >3 METs. They suggest that from the viewpoint of bone health, elderly people should be encouraged to engage in low- and moderate-intensity habitual PA, taking >7,000 steps/day with a duration >15 min/day at >3 METs.[69]

Not all investigations agree that physical activity is positively related to BMD. Kemmler et al.[7] reported on habitual PA and non-athletic exercise in a cross-sectional study of 150 early postmenopausal women (55.5 ± 3.4 years). Activity was assessed by questionnaire and hip BMD was assessed by DEXA. The relationship between PA and BMD measured at the hip was not significant. The authors concluded that habitual PA may not be effective in maintaining bone during and after menopause.[7]

The influence of PAv on hip BMD may be a result of principles underpinning the theory of mechanostat and Wolff's Law. PAv may be an objective measure of bone-muscle interaction and strain rate at the hip. During physical activities like walking or running, external forces act on the hip and produce external torques or moments about the hip. These external moments are met by muscle contractions and peri-articular forces

(i.e., internal moments) to stabilize and lift the body's center of mass producing movement.[70]

In addition to producing the internal moments required for physical activity, muscular and peri-articular forces (i.e., ligaments and capsules) also create repetitive bone strains.[70] Muscular forces and bone loadings have been reported to correlate with bone distribution in normal adults. Furthermore, muscles impart strains to the skeleton during physical activity by producing bone bending moments, as well as compressive and tensile forces. Additionally, hip muscular strength has been significantly associated with hip BMD in postmenopausal women.[70]

The cross-sectional findings of Wang et al.[70] suggest that submaximal muscular loading events, produced during activities of daily living, may influence hip strain history and correlate with bone morphology in postmenopausal women. In Wang's investigation, hip joint moments generated during jogging significantly explained 13–32% of the variance in hip bone mass.[70] Wang et al.[70] reported that physical activity, particularly stair stepping, was significantly associated with hip bone mass in sedentary older women.

In the present study, physical activity intensity was also evaluated using accelerometers. Unlike physical activity volume, women in the Moderate and Vigorous physical activity intensity categories, singularly and combined, did not differ in their likelihood of experiencing significant gains (> 75th percentile) in hip BMD compared to women in the Low PAi category.

The present study is not in agreement with one other epidemiologic investigation that has studied physical activity intensity using accelerometers. Jämsä and colleagues[17]

examined the association between the intensity of PA and BMD changes at the proximal femur, using long-term quantification of daily PA in 64 women between the ages of 35 and 40. The women carried an accelerometer-based movement recorder for 12 months to objectively measure their daily PA. The researchers observed a significant relationship between PA data and proximal femur BMD. Physical activity that induced acceleration levels exceeding 3.6 *g* correlated positively with BMD change at the proximal femur, the association being strongest at 5.7 *g* ($r = 0.42$, $P = 0.001$).[17] They concluded that the association between PA and proximal femur BMD was dependent on the acceleration level or intensity of the exercise.[17]

Physical activity is only one aspect of lifestyle that affects BMD.[7, 42] Cardiorespiratory fitness, indexed by VO_2max, is a function of genetics, anthropometric variables, and many lifestyle factors, including PAv and PAi.[42, 44, 45] The relationship between CRF and BMD is questioned in the literature.[7] Older studies have reported a significant relationship between CRF and BMD,[42, 47] while more recent research has not observed a significant relationship.[46]

Pocock and colleagues[42] studied the relationship between CRF and bone mass in the femoral neck in 84 normal women using a cross-sectional design. BMD was significantly correlated with CRF ($r = 0.60$, $P < 0.001$).[42] In the 45 postmenopausal participants, CRF was significantly correlated with femoral neck BMD ($r = 0.46$, $P < 0.01$).[42] The authors concluded that increased CRF may increase bone mass at the sites of clinically important fractures in osteoporosis.[42]

Chow et al.[47] conducted a cross-sectional study to determine the relationship between bone mineral mass and CRF in active, healthy, postmenopausal women from 50 through 59 years of age. In vivo neutron activation analysis was used to measure calcium or bone mineral in the trunk and proximal femurs. Cardiorespiratory fitness was determined by VO_2max, attained by a GXT on the treadmill. The "above-average fit" group (VO_2max > 29 $mL \cdot kg^{-1} \cdot min^{-1}$) when compared to the "average fit" group (VO_2max 21-29 $mL \cdot kg^{-1} \cdot min^{-1}$) had significantly higher bone mineral ($P < 0.001$). There was a significant correlation between CRF and bone mineral ($P < 0.01$). The authors concluded that level of PA may modify the amount of bone loss in postmenopausal women.[47]

Lastly, a cross-sectional study by Kemmler et al.[7] reported a negative relationship between CRF and BMD in 150 early postmenopausal women ($r = -0.22$, $P < 0.05$). However, this relationship was no longer significant after controlling for potential confounders, which contradicts the findings of the early studies.[7]

In the present study, women with high levels of measured CRF did not differ from those with low levels of CRF regarding the percentage that experienced significant gains (> 75th percentile) in hip BMD from baseline to follow-up. In short, according to the findings of the present study, high and moderate levels of CRF do not appear to increase the likelihood of significant gains in BMD at the hip compared to low levels of CRF over a six-year duration. On the surface, this finding seems to contradict the results of most of the other studies mentioned previously. However, it is important to note that other investigations examining the link between CRF and BMD have employed cross-sectional designs. The present study used a six-year, prospective design. Post-hoc evaluation of the

baseline CRF and baseline hip BMD relationship of the present study revealed significant findings, similar to those reported by Pocock et al. and Chow et al.[42, 47] Specifically, the baseline cross-sectional results showed that with body mass controlled, women in the high CRF category had significantly greater mean hip BMD than women with moderate or low levels of CRF (F=3.51, P=0.01).

Evidently, most investigations, including the present study, show that hip BMD is related significantly to estimated CRF levels using cross-sectional designs. However, significant *gains* in hip BMD are not more common among fit women compared to their counterparts. This may be partly a function of the ceiling effect. That is, because fit middle-aged women tend to have the highest hip BMD levels, it is difficult for these women to gain additional BMD over time. Room for improvement is limited. The natural law of regression to the mean may be working against fit women. Hence, it appears that when viewed prospectively, fit middle-age women do not have an advantage over unfit women regarding gaining significant bone density at the hip.

In the present study, none of the relationships between baseline PAv, baseline PAi, nor baseline CRF, and changes in spine BMD, was statistically significant. Much like the present study's results, Kemmler et al.[7] observed slight relationships between PA and BMD measured at the hip and the spine, but did not detect significant relationships.[7] Unlike the present study, Pocock[42] reported that spine BMD was positively correlated to CRF ($r = 0.54$, $P < 0.001$).

On the contrary, a cross-sectional study by Bidoli[30] et al. using 1373 women (40-64 years) supports the possibility that past and recent physical activity helps protect

middle-aged women's spine BMD. They investigated PA at work and in leisure time for three specific periods of life: age 12, between 15 and 19 years, and between 30-39 or 50-59 years. Leisure time PA at 15-19 years old for low versus high tertiles was not related to lumbar spine BMD (OR: 1.4, 95% CI: 0.8-2.4), but leisure time PA was related significantly to spine BMD when leisure time PA at the most recent age was used (OR: 1.7, 95% CI: 1.1-2.6).[30]

This investigation is not without limitations. First, the sample was relatively homogeneous (e.g., white, female, and non-smokers), which decreases the ability to make broad generalizations. Second, the use of accelerometers provided objective measures of PAv and PAi but did not provide information regarding the mode of physical activity. Third, physical activity was only measured for 7 days. Fourth, regarding PAi, there were few women who engaged in vigorous activity for greater than 150 minutes in 7 days. However, the sample displayed a broad range of PAv and cardiorespiratory fitness.

In summary, the present study is unique in that there are few if any studies that have objectively measured PAv and PAi and described their relationships with changes in hip and spine BMD longitudinally. The present investigation indicates that middle-aged women who have moderate or high levels of physical activity volume are more likely to experience significant gains in hip BMD over time compared to those with low levels of physical activity volume. These gains appear to be independent of age, time in the study, baseline hip BMD, baseline body mass, changes in body mass, average calcium intake, average vitamin D consumption, and changes in perceived fitness. Furthermore, from a cross-sectional viewpoint, women with high levels of physical fitness are significantly

more likely to have greater mean hip BMD levels than women with moderate or low levels of CRF. However, the cross-sectional relationship between CRF and hip BMD does not appear to hold over time, when evaluated using a prospective design. Moreover, according to the present investigation, the relationship between intensity of physical activity and changes in hip BMD is not statistically significant. Lastly, neither of the associations between volume and intensity of physical activity and changes in spine BMD, nor the relationship between CRF level and changes in spine BMD appears to be significant.

Given low hip BMD and high fracture rates are common among American women, based on the results of the present investigation, it is advisable for middle-aged women to strive to engage in moderate to high levels of physical activity volume in order to maximize the likelihood of gaining bone mineral density over time.

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Table 1 Changes in key variables from baseline to follow-up

	Baseline N = 244		**6-years N = 244**		**Change N = 244**			
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***t***	***P***
Mass (kg)	66.1	10.9	68.1	11.5	2.0	5.3	6.0	.0001
Hip BMD (g/cm^2)	0.934	0.114	0.919	0.117	-0.015	0.045	-5.3	.0001
Spine BMD (g/cm^2)	1.016	0.120	1.005	0.133	-0.010	0.054	-2.8	.0006

Variables on rows with significant P-values ($P< 0.05$) indicate that there was significant change from baseline to the follow-up 6 years later.

Table 2 Description of predictor variables and incidence of hip BMD gain

Category	n	Mean	SD	Range	BMD Gain Category (>75th percentile)	χ^2_{mh}
PAv[a]						6.1*
Low	62	1652.1	23.68	0.83-1.95	12.9%	
Moderate	120	2553.1	32.60	1.99-3.08	28.3%	
High	62	3907.7	76.21	3.11-6.64	32.3%	
PAi[b]						0.1
Low	200	1002.2	6.82	0-29,999	20.9%	
Moderate	27	18.6	4.71	30,000-49,999	29.6%	
Vigorous	17	23.2	8.21	$\geq$ 50,000	17.7%	
CRF [c]						1.4
Low	65	28.1	3.64	15.1-31.9	27.7%	
Moderate	114	36.6	2.48	32.0-40.6	28.1%	
High	65	46.0	4.45	41.0-63.0	18.5%	

[a]PAv was measured in total activity counts per week divided by 1,000.

[b]PAi was measured in counts per 10 minute interval and mean number of intervals within a specific category for 7 days.

[c]CRF was estimated in $mL \cdot kg^{-1} \cdot min^{-1}$.

* statistically significant at the p = 0.05 level.

The Low PAv category reflects the lowest quartile of PAv, the Moderate PAv category represents the middle-two quartiles, and the High PAv category reflects the highest quartile at baseline.

The Low CRF category reflects the lowest quartile of CRF, the Moderate CRF category represents the middle-two quartiles, and the High CRF category reflects the highest quartile at baseline.

Table 3 Likelihood of significant hip BMD gains ($\geq 75^{th}$ percentile) in High and Moderate PAv compared to Low PAv women at baseline over 6 years

	Outcome: Increased BMD ($\geq 75^{th}$ percentile)					
	High vs Low PAv		High-Moderate vs Low PAv		Moderate vs Low PAv	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	2.50	1.19-5.24	2.30	1.16-4.56	2.20	1.08-4.45
Age	2.45	1.16-5.18	2.26	1.34-4.50	2.16	1.06-4.40
CRF	3.72	1.45-9.51	2.72	1.34-5.51	2.44	1.18-5.01
Baseline hip BMD	2.50	1.20-5.23	2.32	1.17-4.58	2.24	1.11-4.52
Baseline mass	2.49	1.18-5.25	2.30	1.16-4.56	2.17	1.07-4.39
Mass change	2.52	1.21-5.26	2.28	1.15-4.52	2.16	1.06-4.39
Time in the study	2.26	1.05-4.84	2.12	1.08-4.17	2.05	1.03-4.09
Menopause status	2.15	1.04-4.44	2.06	1.06-4.01	2.02	1.01-4.00
Calcium intake	2.41	1.15-5.04	2.19	1.10-4.33	2.08	1.03-4.21
Vitamin D intake	2.51	1.20-5.27	2.28	1.15-4.51	2.17	1.07-4.39
Perceived fitness change	2.66	1.26-5.63	2.40	1.21-4.77	2.28	1.12-4.62
All of the above	4.08	1.43-11.60	2.45	1.24-4.84	2.20	1.11-4.38

RR=relative risk. Low PAv was the reference category.

95% CI=95% confidence interval.

Low PAv included women in the lowest quartile (reference group: n=62). Moderate PAv included women in the middle-two quartiles (n=120). High PAv included women in the highest quartile (n=62). All of the comparisons were statistically significant.

Table 4 Relationships between physical activity volume and the covariates

	Physical Activity Volume Groups							
	Low	Moderate	High					
Covariate	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	**P**
Baseline hip BMD	0.92 ± 0.11	0.94 ± 0.12	0.93 ± 0.10	0.9	0.42
Age (years)	42.1 ± 3.0	41.5 ± 2.8	41.4 ± 3.4	1.4	0.26
CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	32.9^{a} ± 5.3	36.0^{b} ± 7.1	42.4^{c} ± 6.5	34.1	<0.01
Baseline mass (kg)	66.0 ± 11.2	67.5 ± 11.0	63.6 ± 10.1	2.7	0.07
Mass change (kg)	1.5 ± 6.3	2.6 ± 4.9	1.5 ± 4.6	1.3	0.29
Vitamin D intake	380.3 ± 233.0	353.9 ± 207.7	352.7 ± 199.1	0.4	0.69
Calcium intake	1307.8 ± 508.0	1203.2 ± 498.6	1238.4 ± 571.1	0.8	0.44
Time in the study	2300.9 ± 617.7	2215.4 ± 713.8	2040.9 ± 807.7	2.2	0.12
Decreased fitness (%)	16.1	23.3	29.0	4.0^{*}	0.40
Postmenopausal (%)	25.8	19.1	16.1	8.8^{*}	0.19

Note: "Decreased fitness" and "postmenopausal" are categorical variables. Results for these two variables reflect the percentage of participants who perceived decreases in their fitness level across the study, and the percentage who were postmenopausal.

*Chi-square statistic

Baseline hip BMD was measured in g/cm^2. Time in the study was measured in days. Calcium intake was measured in mg. Vitamin D intake was measured in iU.

Means on the same row with different superscript letters are significantly different ($P < 0.05$).

Table 5 Relationships between physical activity intensity and the covariates

	Physical Activity Intensity Groups							
	Low	Moderate	Vigorous					
Covariate	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	**P**
Baseline hip BMD	0.93 ± 0.11	0.95 ± 0.10	0.92 ± 0.13	0.6	0.53
Age (years)	41.6 ± 3.0	42.1 ± 3.2	40.9 ± 2.8	0.7	0.48
CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	35.5^{a} ± 6.5	39.4^{b} ± 6.7	48.5^{c} ± 6.7	32.9	<0.01
Baseline mass (kg)	66.4^{a} ± 11.1	68.2^{a} ± 10.3	59.1^{b} ± 7.2	4.3	0.01
Mass change (kg)	2.1 ± 5.4	1.1 ± 5.4	2.4 ± 3.0	0.5	0.64
Vitamin D intake	359.3 ± 217.5	355.4 ± 190.1	380.4 ± 182.9	0.1	0.92
Calcium intake	1263.3 ± 519.4	1069.1 ± 526.0	1218.8 ± 492.4	1.7	0.19
Time in the study	2254.4^{a} ± 664.8	1971.0^{b} ± 843.3	1821.0^{b} ± 969.5	4.4	0.01
Decreased fitness (%)	22.5	22.2	29.4	1.4^{*}	0.85
Postmenopausal (%)	21.5	14.8	11.7	8.8^{*}	0.19

Note: "Decreased fitness" and "postmenopausal" are categorical variables. Results for these two variables reflect the percentage of participants who perceived decreases in their fitness level across the study, and the percentage who were postmenopausal.

*Chi-square statistic

Baseline hip BMD was measured in g/cm^2. Time in the study was measured in days. Calcium intake was measured in mg. Vitamin D intake was measured in iU.

Means on the same row with different superscript letters are significantly different ($P < 0.05$).

Table 6 Relationships between cardiorespiratory fitness and the covariates

	Cardiorespiratory Fitness Groups							
	Low	Moderate	High					
Covariate	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	**P**
Baseline hip BMD	0.94 ± 0.10	0.93 ± 0.12	0.94 ± 0.11	0.2	0.79
Age (years)	42.0 ± 2.7	41.7 ± 3.1	41.0 ± 3.1	2.2	0.11
Baseline PAv	21.3[a] ± 6.0	26.1[b] ± 8.3	33.1[c] ± 10.0	33.9	<0.01
Baseline mass (kg)	70.7[a] ± 11.3	66.5[b] ± 10.7	60.9[c] ± 8.6	14.8	<0.01
Mass change (kg)	2.3 ± 6.6	1.6 ± 5.0	2.5 ± 4.1	0.7	0.50
Vitamin D intake	336.2 ± 204.2	348.5 ± 213.6	405.3 ± 212.2	2.1	0.13
Calcium intake	1189.2 ± 498.0	1247.5 ± 503.6	1272.8 ± 571.3	0.5	0.64
Time in the study	2212.6 ± 707.7	2155.3 ± 758.1	2238.8 ± 668.5	0.3	0.73
Decreased fitness (%)	9.2	23.7	35.4	16.6[*]	<0.01
Postmenopausal (%)	24.6	19.3	16.9	8.8[*]	0.19

Note: "Decreased fitness" and "postmenopausal" are categorical variables. Results for these two variables reflect the percentage of participants who perceived decreases in their fitness level across the study, and the percentage who were postmenopausal.

[*]Chi-square statistic

Baseline PAv was measured in counts per 7 days and divided by 100,000. Baseline hip BMD was measured in g /cm^2. Time in the study was measured in days. Calcium intake was measured in mg. Vitamin D intake was measured in iU.

Means on the same row with different superscript letters are significantly different ($P < 0.05$).

Table 7 Relationships between hip BMD change and the covariates

	Hip BMD Change Groups							
	Low		Moderate		High			
Covariate	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**		**F**	**P**
Baseline hip BMD	0.96 ± 0.11		0.92 ± 0.12		0.93 ± 0.11		2.2	0.12
Age (years)	42.8[a] ± 3.0		41.1[b] ± 2.9		41.3[b] ± 3.0		6.8	<0.01
Baseline PAv	26.2 ± 9.6		26.3 ± 9.9		27.9 ± 8.0		0.7	0.50
Baseline mass (kg)	66.2 ± 10.7		65.8 ± 11.1		66.8 ± 10.8		0.2	0.84
Mass change (kg)	0.3[a] ± 5.1		2.6[b] ± 5.7		2.5[b] ± 4.2		4.3	0.01
Vitamin D intake	370.2 ± 209.4		365.5 ± 212.1		340.6 ± 215.5		0.4	0.69
Calcium intake	1291.8[a] ± 510.0		1284.3[a] ± 539.6		1097.6[b] ± 469.4		3.1	<0.05
Time in the study	2480.5[a] ± 335.0		2203.3[a] ± 710.6		1893.8[b] ± 884.0		11.0	<0.01
Decreased fitness (%)	25.0		24.6		17.7		5.4[*]	0.25
Postmenopausal (%)	46.7		14.8		4.8		46.7[*]	<0.01

Note: "Decreased fitness" and "postmenopausal" are categorical variables. Results for these two variables reflect the percentage of participants who perceived decreases in their fitness level across the study, and the percentage who were postmenopausal.

[*]Chi-square statistic

Baseline PAv was measured in counts per 7 days and divided by 100,000. Baseline hip BMD was measured in g /cm^2. Time in the study was measured in days. Calcium intake was measured in mg. Vitamin D intake was measured in iU.

Means on the same row with different superscript letters are significantly different ($P < 0.05$).

Appendix A

Prospectus

Chapter 1

Introduction

Bone is a dynamic tissue which undergoes continuous remodeling with mineral apposition by osteoblasts and resorption by osteoclasts.[1, 2, 8, 71-72] It adapts its structure to the mechanical stresses and strains placed on it.[1, 2, 8, 71,72] Bone remodeling compensates for weakening bone matrix by adding new organic matrix to strengthen the structure.[72] In children, the rates of remodeling are rapid and their bones normally show little brittleness in comparison with the bones of the elderly.[72] With age and menopause in women, bone remodeling becomes dysfunctional, where bone resorption increases and the resorption cavities are not completely filled leading to bone loss.[1, 72]

Age-related deterioration of bone micro-architecture and low bone mass due to bone remodeling dysfunction is called Type II osteoporosis.[1-4] Type I osteoporosis is reduced bone mass due to deficiencies in estrogen because of menopause or ovarian failure in women.[1-4] Secondary osteoporosis of the hip and spine may result from any of several factors including malnutrition, liver and kidney diseases, endocrine disorders, low BMI, and physical inactivity.[1, 2, 5]

Disuse and inactivity lead to bone loss, whereas weight-bearing physical activity (PA) may maintain or improve bone mineral density (BMD) by transmitting mechanical stresses to bone both directly and indirectly.[2, 6, 7, 71] According to Wolff's law, osteoclasts and osteoblasts directly optimize bone mass, geometry, and material properties to withstand habitual loading.[7, 8] The indirect mechanism occurs through bone-muscle

interactions described by the theory of mechonstat.[7] Overtime, greater mechanical loads and muscle-bone interactions cause osteoblasts to deposit more bone mineral matrix to strengthen the bone, which increases its BMD.[2]

Bone's response to load is highly dependent on the magnitude, rate, distribution, and cycles of strain.[7, 8] Different volumes and intensities of PA translate into different magnitudes, rates, distribution and cycles of loads, which appear to cause different levels of strain on bone, initiating different osteogenic responses and different densities.[2, 7, 8, 71] Thus, bone mineral density should be related to an individual's habitual PA pattern.

Physical activity through the fourth decade of life in women is an important determinant of BMD.[2, 5, 6, 9-12] Increasing PA to improve BMD may represent a feasible strategy for primary prevention of each type of osteoporosis.[5, 6, 13-17] However, there are few studies of the effects of physical activity on BMD of the hip and spine in perimenopausal women.[18-21] Furthermore, experimental studies on this relationship in perimenopausal women have inconsistent findings, with a few studies observing gains in BMD, some showing maintenance of BMD, and others revealing loss of BMD.[7, 20, 22-27]

Randomized double-blinded controlled trial is the best research design for making statements about causality, however, this design has difficulty with long term relationships (e,g., the PA and BMD relationship).[28] As a weakness of this type of study, the duration is not long enough to identify meaningful changes in bone.[46] A second threat is that compliance of participants wanes with time. Furthermore, attrition decreases the statistical power of such studies.[46] For example, one study with long-term data, the Erlangen Fitness Osteoporosis Prevention Study, reported 15% attrition after 14 months

and a 29% drop out rate at the 50-month follow-up visit.[13, 46] The study's attendance rate for the remaining exercising participants at the 14-month report was 2.7±0.4 out of four sessions and 2.4 sessions at the 50-month follow-up.[13, 46] In short, only 71% of the study's women were participating only 60% of the time.

Prospective cohort studies reveal clarifying information on habitual physical activity on risk of bone loss.[2, 13, 28] This type of study could be enlightening on the theory that sustained high levels of PA yield greater BMD levels which serve as a reservoir of bone mass and a protection from osteoporosis.[2, 5, 29] Prospective reports may support the hypothesis that PA protects against osteoporosis through increased BMD,[5, 17, 30, 31] "but the quality of the evidence supporting this notion is weak."[19]

The quality of evidence in prospective cohort studies is weak because the PA measurement is typically based on questionnaires and is rarely measured objectively.[7,27, 28] Questionnaires are subject to recall bias, are unable to account for daily PA, and are often inflated accounts of PA.[32-34] Furthermore, existing studies assessed PA most often only by hours per week, which does not quantify physiologic strain frequencies.[7] The adaptive response of bone is sensitive to strain number, strain duration, strain intensity and impact, but more so the latter two.[7] Thus, to quantify the influence PA has on BMD, volume and intensity of PA should be considered.[7, 30, 35, 36]

Accelerometers can objectively measure volume of PA over a given time period (e.g., 7 days).[17, 37, 38] Furthermore, intensity of PA can be deduced by dividing volume of PA by a specific time or epoch (e.g., 10 minutes). Thus, variations in activity counts

within a specific time period reflect differences in PA intensity (PAi).[39-41] Thus, volume and intensity of PA can be measured objectively.

Physical activity is only one aspect of lifestyle that affects BMD. [7, 42] Cardiorespiratory fitness (CRF), indexed by VO_2max, is a function of PA volume (PAv), PAi, and percent body fat, which all correlate with BMD.[42, 44, 45] However, the relationship between CRF and BMD is questioned in the literature.[46] Older studies have reported a significant relationship between CRF and BMD,[42, 47] while more recent research has not observed a significant relationship.[46]

The theory supporting the CRF-BMD relationship is that CRF is a surrogate of training status or habitual intense PA, various lifestyle factors, and mechanical load placed on the skeleton.[42] Thus, the two should be positively correlated. Furthermore, an individual's CRF level should reveal the nature of an adult's long-term PA, because CRF is significantly related to habitual PAi.[42, 45]

Bone mineral density is associated with the habitual PA and lifestyle factors of young women, but this relationship is not well supported in perimenopausal females.[2, 6, 9-11, 18-21] The theory behind this relationship is plausible and prospective cohort studies are a good source for evidence of the PA/CRF-BMD relationship.[2, 13, 28] However, PA is rarely measured objectively using accelerometers.[7, 27, 28] Therefore, a prospective cohort design studying the relationship between PA using accelerometers, and BMD measured by DEXA, would be a worthwhile addition to the body of literature on osteoporosis prevention. Furthermore, clarification of the relationship between CRF, indexed by VO_2max, and BMD is warranted because of the inconsistencies found in the literature.

Statement of the Problem

The purpose of this prospective cohort study will be to combine measurements of objectively measured PAv, objectively measured PAi, CRF, indexed by VO_2max, and BMD, using dual energy x-ray absorptiometry, to evaluate the risk of BMD loss and osteoporosis in perimenopausal women over a seven-year period. A secondary purpose will be to ascertain the extent to which age, dietary intake, baseline BMI, changes in BMI, baseline BMD, maternal history of osteoporosis, and menopause status influence these risks over time.

Research Questions

1. To what extent do perimenopausal women with high, moderate, and low levels of physical activity volume differ in risk of BMD loss at the hip and spine over a seven-year duration?
2. To what extent do perimenopausal women with high, moderate, and low levels of physical activity volume differ in risk of developing osteoporosis of the hip and spine over a seven-year duration?
3. To what extent do perimenopausal women with high, moderate, and low levels of physical activity intensity differ in risk of BMD loss at the hip and spine over a seven-year duration?
4. To what extent do perimenopausal women with high, moderate, and low levels of physical activity intensity differ in risk of developing osteoporosis of the hip and spine over a seven-year duration?

5. To what extent do perimenopausal women with high, moderate, and low levels of cardiorespiratory fitness differ in risk of BMD loss at the hip and spine over a seven-year duration?
6. To what extent do perimenopausal women with high, moderate, and low levels of cardiorespiratory fitness differ in risk of developing osteoporosis of the hip and spine over a seven-year duration?
7. To what extent does dietary intake, age, baseline BMI, changes in BMI, baseline BMD, maternal history of osteoporosis, and menopause status influence these risks?

Assumptions

1. Participants wore their accelerometers properly and at all times during baseline data collection, except when engaged in water activities.
2. Participants engaged in "typical" physical activity during the week of observation at baseline.
3. Participants honestly responded to the Physical Activity and Lifestyle Questionnaires, Block Food Frequency Questionnaires, and Eating Behavior Questionnaires at baseline and will respond honestly to them again at the 7-year follow-up assessment.

Delimitations

1. Participants will be middle-aged women living on the Wasatch Front who participated in the BYU Lifestyle Project in 2000.
2. All participants were premenopausal at baseline.

3. All participants were nonsmokers and nonobese (< 30 BMI) at baseline.
4. BMD was measured at baseline and will be measured again at the 7-year follow-up using a Hologic QDR 4500 W bone densitometer (Hologic, Waltham, MA).
5. Physical activity was measured using CSA model 7164 accelerometers (Actigraph, Pensacola, FL) at baseline.
6. Diet was measured using the Block food frequency questionnaire at baseline.

Limitations

1. The results of this study may not be generalized to all races due the homogenous participant population.
2. A prospective cohort design is less effective than a randomized controlled experimental study at satisfying the principle of "association" required by the Bradford Hill criteria for making statements about causation.

Operational Definitions

Bone Density-Quantitative measurement of the mineral content of bone, used as an indicator of the structural strength of the bone and as a screen for osteoporosis.

Bone Mineral Density (BMD)-Measurement of the amount of mass bone, typically expressed in g/m^2.

Bone Mineral Density Loss-Loss of bone mass, typically expressed in negative g/m^2.

Bone Remodeling-Continuous mineral deposition by osteoblasts and absorption by osteoclasts.

Cardiorespiratory Fitness (CRF)-A set of attributes, primarily respiratory and cardiovascular, relating to the ability to perform work requiring expenditure of energy.

Osteogenic Response-Formation of bone due to a stimulus (or stimuli).

Osteoporosis-Significant reduction in the quantity of bone or atrophy of skeletal tissue, specifically a T score of -2.5 or lower.

Physical Activity (PA)-Bodily movement produced by skeletal muscle contraction that requires energy expenditure. Characterized by features including frequency, intensity, duration, and type.

Physical Activity Intensity (PAi)-How hard a person exerts during physical activity expressed as variations in accelerometer activity counts within a specific time period (e.g. 10 minutes).

Physical Activity Volume (PAv)-The total number of activity counts completed during a week's time.

Bone Resorption-The loss of substance from bone by lysis, or by physiologic or pathologic means.

Chapter 2

Review of the Literature

Bone Mineral Density and Osteoporosis

Osteoporosis is a disease that begins by failure to achieve an optimal peak bone mass. Peak bone mass or peak bone mineral area density is the amount of bone tissue present when skeletal maturation is completed. The term relates to the maximal bone mass accumulation that an individual is able to achieve, given optimal intrinsic and extrinsic factors that determine peak bone mass.[73, 74] The intrinsic factors are not modifiable and include race, gender, heredity, bone structure, menopause/menstrual history, and chronic illnesses.[73-75] The extrinsic factors, which may be modified, are diet, physical activity, body weight, hormone levels, and other lifestyle practices, such as smoking and medications.[75] Smaller bone structure, hormonal changes, and several lifestyle factors predispose women to bone loss, increasing their risk of developing osteoporosis.[77] Individuals with several risk factors have an increased risk for developing osteoporosis by compromising their peak bone mass.[4]

Peak bone mass is determined early in life with approximately 45% of peak bone mass achieved during the 2 years of the adolescent growth spurt.[3, 73] Nearly 98% of peak bone mass has accrued by age 18, but the exact age at which bone accumulation reaches a plateau varies with skeletal region.[73, 76]

Cooper et al.[77] reported on the relationships between childhood growth, lifestyle, and peak bone mass in 153 women born during 1968-1969. They traced the growth of these women during childhood using birth and school health records. In 1990, the authors

measured BMD using dual energy x-ray absorptiometry (DEXA). They reported PA was the major lifestyle determinant of BMD after allowing for body build. They concluded, "Growth primarily determines the size of the skeletal envelope, and its trajectory is established by age 1 year. Activity, in contrast, modulates the mineral density within the skeletal envelope and may contribute to the consolidation of bone following the end of linear growth."[77]

The results of the Drake et al.[78] study suggest that bone mass may accrue in certain groups of women beyond adolescence. The researchers followed 164 healthy, young Caucasian female midshipmen at the United States Naval Academy for 3.6 years.[78] Over the study period, hip BMD increased 2.26% ($P < 0.001$), lumbar spine (LS) BMD increased 3.27% ($P < 0.001$) and distal tibia BMD increased 5.2% ($P < 0.001$). Total body bone mineral content showed a 5.25% ($P < 0.001$) increase during the study period.[78] In this group of young women, gains in BMD and total body mineral content continued until at least the age of 22. The authors of this study commented, "The significance of this increase in bone mass during early adulthood on risk for osteoporotic fractures in later life and its impact on exercise-related bone injuries are unknown and warrant further examination."[78]

Physical Activity

Physical activity is a major determinant of BMD. For this review of the literature, PA will include body movement produced by muscles that results in energy expenditure,[54] except for resistance training. Furthermore, this review will be delimited to studies that include findings on the BMD of the hip and spine of women.

Experimental Studies on Exercise and Bone Mineral Density

The results of a meta-analysis suggest that aerobic exercise helps to maintain LS BMD in postmenopausal women. This meta-analysis with a total of 17 effect sizes consisting of 330 postmenopausal women (192 exercise, 138 nonexercise) from 10 studies reported on aerobic activity and changes in LS BMD. [79] The primary outcome measure of interest was the treatment effect defined as the percent change in lumbar spine bone mineral density, calculated by subtracting the percent change in the exercise group (EG) from the percent change in the non-training controls (CG). [79] A fixed effects model showed significant changes in lumbar spine bone mineral density (2.83 ± 0.77% (mean ± SD), 95% confidence interval (CI) = 1.33 to 4.35%).[79] The overall average increase was caused primarily by the loss of lumbar spine bone mineral density in the nonexercise group relative to the exercise group (EG = .32 ± 2.46%, 95% CI = -0.94 to 1.58; CG = -2.51 ± 2.69%, 95% CI = -4.60 to -0.96).[79]

A second meta-analysis examined the effects of exercise on regional BMD in postmenopausal women. A total of 11 randomized trials yielding 40 outcome measures and a total of 719 postmenopausal women (370 exercise, 349 nonexercise) were analyzed separately and sample weighted decreases of approximately -0.51% and -0.86% were found for the EG and CG, respectively.[22] Aerobic training enhanced regional BMD (mean change: aerobic, 1.62% and 95% CI = 1.12-2.12).[22] The meta-analytic review of included studies concluded that exercise may slow the rate of bone loss in postmenopausal women.

Intensity of an exercise program seems to play a key role in the accrual of BMD. In an exercise trial of osteopenic women, the researchers emphasized low-volume high-resistance strength training and high-impact aerobics. Forty-eight fully compliant women (55.1±3.3 years) with no medication or illness affecting bone metabolism participated in an EG and 30 women (55.5 ± 3.0 years) served as the CG.[24] The training consisted of two group-training and two home-training sessions per week. The study participants of both groups were individually supplemented with calcium and vitamin D. The researchers measured BMD using DEXA at the LS.[24] After 38 months, the EG's LS BMD increased 0.8% (not significant) and the CG's decreased by -3.3% ($P < 0.001$).[24] Between-group difference relative to the EG were for the LS was 4.1% ($P < 0.001$).[24] The authors summarized that a low-volume/high-intensity exercise program was successful maintaining BMD at the spine in osteopenic women.[24]

A randomly selected population-based sample of 120 women from a cohort of 5,161 women, aged 35 to 40 years, assessed the effects of high-impact exercise on the BMD of premenopausal women at the population level.[25] The exercise regimen consisted of supervised, progressive high-impact exercises three times per week and an additional home program for 12 months.[25] The exercise group demonstrated significant change compared with the CG in femoral neck BMD (1.1% vs. -0.4%; $P = 0.003$), intertrochanteric BMD (0.8% vs. -0.2%; $P = 0.029$), and total femoral BMD (0.1% vs. -0.3%; $P = 0.006$).[25] No exercise-induced effects were found in the total lumbar BMD or in the lumbar vertebrae L2-L4. Instead, L1 BMD (2.2% vs. -0.4%; $P = 0.002$) increased significantly more in the EG than in the CG.[25] This study indicates that high-impact

exercise is effective in improving BMD in the LS and upper femur in premenopausal women and this type of training may be an efficient, safe, and inexpensive way to prevent osteoporosis later in life.[25]

Chan et al.[80] evaluated the efficacy of calcium supplementation and weight-bearing exercise in reducing rate of bone loss in postmenopausal Chinese women in a randomized controlled trial. They randomly assigned 205 postmenopausal Chinese women (59 ± 3 years) to CG (n = 100), calcium (1200 mg calcium carbonate, n = 70) or calcium-EG (4 hours of brisk walking per week in addition to 1200 mg calcium carbonate, n = 35) for 24 months. They measured BMD at baseline and subsequently every six months for two years. The one-way within-subjects ANOVA analysis indicated that the CG experienced significant bone loss at all the skeletal sites ($P < 0.05$).[80] There was no significant bone loss for either the calcium or calcium-EG. Using ANOVA repeated measures, the percentage of bone loss in the CG was significantly higher when compared to the calcium or calcium-EG, at the total body (CG -0.77%, calcium -0.14%, calcium-EG +0.37%; $P < 0.05$), LS L2-L4 (CG -0.74%, calcium 0.34%, calcium-EG +0.69%; $P < 0.05$), femoral neck (CG -1.24%, calcium +0.90%, calcium-EG +2.62%; $P < 0.05$) and total hip (CG -2.21%, calcium -0.26%, calcium-EG +2.24%; $P < 0.05$).[80] The mean percentage change in BMD in the calcium-EG was significantly different from the calcium group at the femoral neck and total hip but not at the total body or LS.[80] The average daily duration of exercise was positively correlated with the changes in BMD at the femoral neck ($r = 0.83$, $P < 0.001$).[80] They concluded adequate calcium intake and

regular moderate exercise were effective in reducing rate of bone loss in postmenopausal women.[82]

Experimental Study using Accelerometers

Vainionpää and colleagues evaluated the association between the intensity of exercise and BMD in a 12-month population-based trial with 120 women (35-40 years) randomly assigned to an EG or to a CG.[36] The researchers assessed intensity of the PA of 64 women with an accelerometer-based body movement monitor.[36] They analyzed daily activity at five acceleration levels (0.3-1.0 g, 1.1-2.4 g, 2.5-3.8 g, 3.9-5.3 g, and 5.4-9.2 g).[36] They assessed BMD at the hip, LS, and radius using DEXA and the calcaneus with quantitative ultrasound.[36] They reported that PA-induced acceleration levels exceeding 3.9 g correlated positively with BMD change in the hip area ($P < 0.05$).[36] L1 BMD change correlated positively with activity exceeding 5.4 g ($P < 0.05$) and calcaneal speed of sound with the level of 1.1-2.4 g ($P < 0.05$).[36] Baseline BMD was negatively associated with BMD change at the hip.[36] They concluded that the intensity of exercise, measured as the acceleration level of PA, was significantly correlated with BMD changes.[36] Furthermore, bone stimulation is reached during normal physical exercise in healthy premenopausal women and the threshold level for improving BMD is less than 100 accelerations per day at levels exceeding 3.9 g in the hip area.[36]

Exercise-Induced Bone Mineral Density Maintenance Study

Exercise is recommended to enhance bone health, but data on the maintenance of exercise-induced bone benefit is sparse. Thus, one study assessed maintenance of the musculoskeletal benefits obtained in an 18-month intervention of high-impact exercise in

premenopausal women (34 former trainees and 31 controls).[26] BMD was measured at baseline, after 18 months, and after 5 years. Changes in BMD in both former trainees and controls were similar at the 18 month and 5 year measurements.[26] The exercise-induced BMD gain (i.e. the mean statistically significant intergroup differences of 1-3% in favor of the trainees) was maintained at the femoral neck at the 5-year follow-up.[26] At the LS, the difference was 1.7% at both 18-month and at the 5-year follow-up, but the between-group difference was not statistically significant in the latter follow-up.[26] The measured regional BMD increased in response to the 18-month intervention and maintenance of this gain was demonstrated at the 5 year follow-up.[26] The findings suggest that long-term bone benefits exist for women who accrue BMD through high-impact training.

Evidence that exercise may lead to an increase in the mineral density of the bones is mounting.[81] However, ACSM and the CDC are recommending more moderate PA, not necessarily planned repetitious exercise. Furthermore, experimental studies have difficulty with long term relationships due to high attrition when experiments last longer than a few months. Few interventional studies on this relationship are controlled for more than six months, which is not typically sufficient for meaningful changes in bone. [28, 46] Similar to the study on BMD maintenance, prospective studies may be better suited for investigating the PA and BMD relationship.

Cross-Sectional Studies Using Questionnaires

Bone mineral density in women has been studied extensively using cross-sectional studies based on questionnaires. The conclusions have been mixed. For example, Ho et al.[82] concluded that PA was associated with bone mass in women

between the ages of 21 and 30, but not between 31 and 40 years. This conclusion was made after they evaluated the role of moderate PA on bone mass around the period of peak bone mass attainment in a cross-sectional analysis of the baseline data of a longitudinal study of 273 women. The researchers analyzed two age groups (21-30 and 31-40 years) and controlled for age, dietary calcium intake, and lean body mass on BMD.[82] The total metabolic equivalent values of leisure time PA was based on the MET values for each activity and the reported time spent on each activity in the past year.[82] The results indicated that among the younger group of women, a high level of leisure time PA was associated with higher bone mass at both the LS and the hip, but not in the older group.[82] Additive effects of PA and dietary calcium intake on the spine and the hip BMD were observed.[82] Together with age and lean body mass, PA and dietary calcium intake accounted for 19% of the variances of BMD at the LS and 9-11% at the hip.[84]

Uusi-Rasi et al., on the contrary, concluded that observed patterns of PA are feasible targets for the primary prevention of osteoporosis.[16] The researchers had screened 422 women in three age groups (25-30, 40-45, and 60-65 years) and divided them into groups by their level of PA.[16] Total body bone mineral content, BMD of the femoral neck and distal radius were measured with DEXA.[16] BMD of the femoral neck was 5% higher in the high PA groups than in the low PA groups.[16] High PA was related to "larger and mechanically more competent bones in the femoral and radial shafts with the association being stronger with increasing age."[16] Results from this cross-sectional study suggest that moderate PA, if maintained throughout menopause, can result in "considerable long-term improvement in the mechanical competence of the skeleton."[16]

The Southern European cross-sectional study supports the possibility that past and recent physical activity helps protect middle-aged women against osteoporosis. The researchers measured BMD by means of dual photon absorptiometry at the LS, and assessed levels of past and recent PA by means of a population-based screening on 1373 women (40-64 years).[30] They investigated PA at work and in leisure time for three specific periods of life: at age 12, between 15 and 19 years, and between 30-39 or 50-59 years.[30] After controlling for other known contributory factors in the development of osteoporosis, they compared low versus high BMD tertiles (i.e., 458 and 461 women, respectively).[30] Leisure time PA and LS BMD were positively associated with trends at age 15-19 (odds ratio (OR) for low versus high tertiles of leisure time activity: 1.4, 95% CI: 0.8-2.4) and at most recent age (OR: 1.7, 95% CI: 1.1-2.6).[30]

Neville et al. demonstrated the importance of high peak strain activities in determining peak bone status in young men, but failed to observe this association in women.[12] The aim of this study was to determine the extent to which different components of PA are related BMD in 242 men and 212 women (20-25 years) by DEXA in the LS and femoral neck.[12] PA was assessed by a self-report questionnaire designed to measure the frequency and duration of PA and its components.[12] Height, weight, diet, and smoking habits were also assessed as potential confounding factors.[12] Sports activity and peak strain sports activity undertaken by men were strongly associated with both LS BMD, LS BMC, and femoral neck BMD and BMC ($P < 0.01$).[12] In women, there were no associations between bone measurements and any component of PA.[12] Sports activity explained 10.4% of the observed variance in LS BMD in men, but < 1% in women.[12] The

authors concluded that these results demonstrate the importance of sports activities, especially those involving high peak strain, in determining peak bone status in young men.[12] They felt that the failure to observe this association in women reflects their lower participation in such activities.[12] However, women may have the same capacity to benefit from these activities as men.[12]

To describe familial relationships among BMD and PA, Runyan et al. enrolled a cohort of 72 early-adolescent daughter-premenopausal mother pairs with 22 postmenopausal maternal grandmothers.[59] The researchers assessed BMD of the hip and LS using DEXA, body height and weight, menstrual function, and current and past PA patterns using questionnaires.[59] Physical activity was a strong predictor of BMD for daughters and mothers.[59] Using cross tabulation to evaluate familial associations comparing one type of aggregate to familial aggregates of lifestyle variables, daughter-mother-grandmother triads with low PA had low femoral neck BMD, whereas those with high PA had high femoral neck BMD ($P < 0.001$).[59] The authors concluded that making PA a part of a daily routine is an important goal for maintaining or improving bone health for women of all ages.[59]

Nevill and colleagues compared the levels of, and the correlation between, BMD recorded at 10 sites in female endurance runners and investigated possible determinants responsible for any inter-site differences observed.[83] ANOVA and factor analysis identified systematic differences in BMD between sites with the greatest BMD being observed in the lower-body sites, in particular the legs.[83] Using ANCOVA the researchers identified that running further distances resulted in higher bone mass in the legs. In

contrast, training for additional years appeared to result in lower bone mass in the arms and lumbar spine.[85] They concluded that running appears to stimulate the bone mass of women endurance runners at lower-body sites.[85]

Recently, Kemmler et al. reported on habitual PA and non-athletic exercise in a cross-sectional study of 150 early postmenopausal women (55.5 ± 3.4 years).[7] Activity and weight-bearing activity were assessed by questionnaire. DEXA, quantitative computed tomography (QCT), quantitative ultrasound (QUS), quantitative ultrasound index (QUI), SOS, and BUA assessed the bone parameters.[7] The weight bearing intensity index had a slight relationship with QUI calcaneus ($r = 0.21$, $P < 0.05$).[7] They observed slight relationships between PA and BMD measured at the hip and the spine, but did not detect significant relationships.[7] The authors concluded that habitual PA may not be effective in maintaining bone during and after menopause.[7]

Cross-sectional Studies Using Accelerometers

The mixed conclusions may be due to the measurement of PA. The use of an objective tool will produce more consistent results. The studies using accelerometers to measure PA have identified positive associations between PA and BMD.

Hasselstrøm et al.[84] assessed the association between objectively measured habitual PA and calcaneal and forearm BMD in 297 boys and 265 girls (6-8 years) using DEXA and an accelerometer during two weekdays and a weekend. The researchers evaluated different definitions of vigorous PA in order to establish thresholds (counts/minute) for bone-stimulating PA.[84] Both calcaneal and forearm BMD were significantly related to total time of daily PA as well as with intense PA above all the

chosen cut-off points (all $P < 0.05$).[84] The beta value for mean counts/minute physical activity was significantly lower than that for all the chosen cut-off points of vigorous activity both for calcaneal and distal forearm BMD.[84] This study suggests that "both habitual daily physical and amount of vigorous physical activity in children aged 6-8 years are associated with appendicular bone mineral density."[84]

Tobias and colleagues examined the relationship of habitual levels of PA on BMD in children by studying the relationship between accelerometer recordings and DEXA parameters in 4457 11-year-old children. They analyzed associations between amount of moderate and vigorous PA (MVPA), derived from accelerometer recordings for a minimum of 3 days, and parameters obtained from total body DEXA scans. The relationship of different activity intensities was also studied by stratification based on lower and higher accelerometer cut-points for moderate (3600 counts/minute) and vigorous (6200 counts/minute) activity, respectively. After adjusting for age, sex, socio-economic factors, and height, with or without additional adjustment for lean and fat mass—the full model, MVPA was positively associated with lower limb BMD and BMC adjusted for bone area ($P < 0.001$).[85] Lower limb BMC was positively related to MVPA after adjusting for height and lean and fat mass ($P < 0.001$), whereas little relationship was observed after adjusting for height and lean mass alone ($P = 0.1$).[85] Using the fully adjusted model, moderate PA exerted a stronger influence on lower limb BMC compared with light activity (light activity: 2.9 [1.2-4.7, $P = 0.001$] and moderate activity: 13.1 [10.6-15.5, $P < 0.001$]).[85] Physical activity was positively related to both BMD in the

adjusted model.[85] They concluded that habitual levels of PA in 11-year-old children are related to BMD, with moderate PA exerting the strongest relationship.[85]

Jämsä and colleagues examined the association between the intensity of PA and BMD at the proximal femur, using long-term quantification of daily PA in 64 women between the ages of 35 and 40.[17] The women carried an accelerometer-based body movement recorder for 12 months to objectively measure their daily PA.[17] The average distribution of daily accelerations was defined using 33 acceleration levels.[17] The researchers observed a significant relationship between PA data and proximal femur BMD.[17] Physical activity that induced acceleration levels exceeding 3.6g correlated positively with the BMD change at the proximal femur, the association being strongest at the femoral neck at 5.7 g ($r = 0.416$, $P = 0.001$).[17] They concluded that the association between PA and proximal femur BMD was dependent on the acceleration level of exercise.[17]

Park and colleagues determined associations between bone health and the quantity and quality of habitual PA in a cohort of 76 men and 96 women (65-83 years). [69] A specially adapted accelerometer measured the number of steps taken and the intensity of physical activity every 4 seconds throughout each 24-hour period for 1 year and distinguished up to 11 levels of PA expressed in METs. At the end of the year, a quantitative ultrasonic technique assessed each participant's osteosonic index (OSI). [69] The data were significantly described by linear and exponential regression models which showed that in both sexes the OSI score increased with increasing daily PA, up to the observed maximum values of approximately 14,000 steps/day and 50 min/day at an

intensity >3 METs. [69] Multivariate-adjusted logistic regression analyses predicted that men and especially women who engaged in < 6,800 steps/day and < 16 min/day of moderate-intensity PA were, respectively, 4.9-8.4 and 2.2-3.5 times more likely to sustain fractures than those participating in >8,200 steps/day and >25 min/day of activity >3 METs. [69] They suggest that from the viewpoint of bone health, elderly people should be encouraged to engage in low- and moderate-intensity habitual PA, taking >7,000 steps/day with a duration >15 min/day at >3 METs.[69]

Possibly due to the objective measurement of PA, findings from cross-sectional investigations using accelerometers are unambiguous, showing that PA is significantly related to BMD. However, cross-sectional studies cannot be used to detail the influence PA has on BMD. Therefore, prospective cohort studies are advantageous to cross-sectional studies.

Prospective Cohort Studies and Bone Mineral Density

Using DEXA in the 1990s, Goto et al.[68] examined whether BMD of the LS and the proximal femur was maintained in pre-, peri-, and postmenopausal women by regular exercise in a longitudinal study. Twenty-six Japanese women (mean age 47.8 years) were followed 4-5 years. Twenty-two participants from volleyball or jogging clubs had participated in the same exercise for more than 5 years at the initial BMD measurement. Longitudinally, for the 22 athletes, the rate of change per year in BMD of the LS was -0.17% in the pre-menopause group and -2.60% in the peri-menopause group.[68] In the proximal femur of the athletes, BMD increased (rate of increase per year 1.80%) in the pre-menopause group, but decreased (rate of decrease per year 1.07%) in the peri-

menopause group.[68] In the pre-menopause group, BMD of the proximal femur increased in all athletes. However, in the proximal femur, the nonexercise group showed a 0.31% decrease, a significant difference ($P < 0.05$) compared with the athletes.[68] The authors suggest that women can achieve continuous gains in bone mass in the proximal femur before menopause by regular intense PA.[68] However, continued high-level PA in this cohort of perimenopausal women was not able to prevent bone loss.[68]

A prospective cohort study reassessed a group of young women (27.8 ± 1.0 years), who previously participated in a placebo-controlled 2-year calcium intervention study at a mean age of 18.5 ± 0.3 years.[31] The researchers observed an early decline in BMD at the neck of the femur (-3.3%/decade) and a converse gain in BMD at the LS (+4.3%/decade) and intertrochanter (+1.9%/decade).[31] This observation suggests site-specific changes in BMD in young premenopausal women.[31] Lifestyle factors were ascertained by questionnaire and a positive association was observed between PA and change in BMD at the total hip and intertrochanter sites ($P < 0.05$).[31] Mein et al[31] summarized the results indicating that PA assists in maintenance of BMD at some sites and thus may contribute to lifelong fracture prevention and maximizing premenopausal BMD is an important strategy for the prevention of osteoporosis and resultant fractures later in life.

Barnekow-Bergkvist and colleagues[5] assessed the relationship between PA, endurance, and muscular strength in 204 randomly selected female students (16.1 ± 0.3 years; range 15–17 years) in 1974. Twenty years later, they reassessed BMD of 36 women of the original 204. They observed that women who were members of a sports

club in adolescence had significantly higher adult BMD (mean differences of 5% to 17% depending on site) compared with participants who were not engaged in a sports club.[5] Furthermore, women with persistent weight-bearing activity in adulthood had significantly higher BMD compared with women who had stopped being active or had never been active.[5] The differences in BMD ranged between 5% and 19% with the highest difference found in trochanter BMD.[5] Membership in a sports club at baseline was a significant independent predictor of BMD in the total body, LS, legs, trochanter, and femoral neck, explaining 17–26% of the variation in BMD.[5] Change in body weight was a strong independent predictor of BMD of the total body and arms explaining 8% of the variation in both sites.[5] In addition, running performance at baseline was an independent predictor of total body BMD of the legs and trochanter.[5] They concluded membership in a sports club and site-specific physical performance in adolescence together with changes in body weight were significantly associated with adult BMD in premenopausal women.[5] Thus, adolescence PA and sustained adult PA contributed to the BMD reservoir in premenopausal women.

According to the prospective cohort studies, premenopausal PA is a significant predictor of BMD in perimenopausal women, but may not prevent the loss of bone after menopause. However, few prospective cohort studies have been conducted and none of the reported studies have used objectively measured physical activity as the predictor variable. Furthermore, the reviewed studies have reported a need exists for additional, well designed longitudinal studies on this topic before a recommendation can be made

regarding the efficacy of PA for maintaining and/or increasing regional BMD and the prevention of osteoporosis in women.[27]

Cardiorespiratory Fitness and Bone Mineral Density

Physical activity is only one aspect of lifestyle that affects BMD.[7, 42] Cardiorespiratory fitness (CRF), indexed by VO_2max, is a function of lifestyle factors including PA volume (PAv), PAi, and percentage body fat, which all correlate with BMD.[42, 44, 45] The relationship between CRF and BMD is questioned in the literature.[46] Older studies have reported a significant relationship between CRF and BMD,[42, 47] while more recent research has not observed a significant relationship.[46]

The first demonstrations of a correlation between CRF, and, by implication, habitual PA, and bone mass in the femoral neck were in 1986. Pocock and colleagues studied the relationship between CRF and bone mass in the femoral neck, LS, and forearm in 84 normal women.[42] A Lunar DP3 dual photon absorptiometer estimated femoral neck and LS BMD.A submaximal cycle ergometer test was used to predict maximal oxygen uptake according to the Astrand and Ryhming criteria.[42] Femoral neck and LS BMD were significantly correlated with CRF ($r = 0.60$, $P < 0.001$).[42] In the 45 postmenopausal participants, CRF was the only significant predictor of femoral neck BMD ($r = 0.46$, $P < 0.01$), and both weight and CRF predicted the LS BMD.[42] The authors concluded that increased CRF may increase bone mass at the sites of clinically important fractures in osteoporosis supporting the correlation between LS BMD and PA.[42]

In 1986, Chow et al. conducted a study to determine if bone mineral mass was influenced by the level of CRF in active, healthy, postmenopausal women from 50 through 59 years of age.[47] In vivo neutron activation analysis (NAA) was used to measure calcium or bone mineral in the trunk and proximal femurs.[47] The NAA measurement is expressed as calcium bone index (CaBI), which relates the participant's Ca value to the estimated mean value for normal participants of the same size based on height and arm span.[47] The normal CaBI is 1.00 ± 0.12 . Level of CRF was determined by VO_2max, attained by a GXT on the treadmill.[47] The "above-average fit" group (VO2max > 29 ml/kg/min) when compared to the "average fit" group (VO_2max 21-29 ml/kg/min) had significantly higher CaBI ($P < 0.001$).[47] There was a significant correlation between VO2max and CaBI ($P < 0.01$).[47] The authors concluded that level of PA may modify the amount of bone loss in postmenopausal women.[47]

Blumenthal and colleagues[86] assigned 101 men (n = 50) and women (n = 51) (mean age = 67.0) in a randomized controlled trial to either an aerobic exercise condition, non-aerobic yoga, or a wait list CG for 4 months to determine the effects of up to 14 months of aerobic exercise on measures of BMD in older adults. Aerobic fitness and bone density were evaluated in all participants at baseline and after 4 months.[86] A semi-crossover design was utilized with all participants completing 4 months of aerobic exercise, followed by another evaluation.[86] All participants were then given the option of 6 additional months of aerobic exercise, after which they had a fourth evaluation.[86] The exercise program included stretching, cycle ergometry, and walking three times per week for 60 minutes throughout the course of the study.[86] A cycle ergometry test assessed

VO_2max and single photon absorptiometry measured BMC.[86] Participants achieved a 10%-15% increase in VO_2max after 4 months of exercise training, and 1%-6% further improvement with additional training.[86] Aerobic fitness was associated with significant increases in BMC in men, but not women, who maintained aerobic exercise for 14 months.[86]

The cross-sectional study by Kemmler et al.[46] also reported on CRF in the same 150 early postmenopausal women. Cardiorespiratory fitness had a slight negative relationship with BMD total hip ($r = -0.22$, $P < 0.05$). However, this relationship was no longer detected after controlling for potential confounders, which contradicts the findings of the early studies.[46] Therefore, further investigation into the relationship between CRF and BMD is warranted.

Conclusion

Experimental studies support the theory that exercise may lead to an increase in women's BMD of the hip and spine. [81] However, experimental studies may not last long enough to observe meaningful changes in BMD. Furthermore, some long-term experimental studies have low statistical power due to attrition.[13, 46]

Many cross-sectional studies on the relationship between PA and BMD have ambivalent findings, which may be due to their inherent weaknesses. However, those studies using accelerometers to objectively measure PA had positive and unequivocal findings. Therefore, the use of accelerometers in a prospective cohort study is recommended.

Only a few prospective cohort studies have been conducted and the reviewed studies have reported a need exists for additional, well designed longitudinal studies on this topic before a recommendation can be made regarding the efficacy of PA for maintaining and/or increasing regional BMD and the prevention of osteoporosis in women.[27] Furthermore, dietary intake, age, initial BMI, changes in BMI, baseline BMD, maternal history of osteoporosis, and menstrual history, should be controlled statistically due to their influences on the relationship between PA and BMD.[5, 31, 44, 80, 82, 83, 85, 87]

The theory supporting the CRF-BMD relationship is that CRF is a surrogate of training status or habitual intense PA and various lifestyle factors.[42] Thus, the two should be positively correlated, especially with body weight controlled. Furthermore, an individual's CRF level should reveal the nature of an adult's long-term PA, because CRF is significantly related to habitual PAi.[42, 45] Therefore, the literature needs more conclusive findings from well-designed, long-term studies investigating the association between CRF, BMD, and risk of osteoporosis.

Chapter 3

Methods

The methods chapter will be divided into five sections: participants, design, procedure, instrumentation and measurement methods, and statistical analysis.

Participants

In 1998, a total of 275 women were recruited using advertisements distributed throughout more than 20 cities in the Mountain West. The participants' mean age was 40.1 $\pm$ 3.0 ($\pm$SD) years. The sample was approximately 90% white, 80% married, and 37% college-graduates. As selection criteria, participants were nonsmokers, nonobese (BMI < 30 kg/m^2), and apparently healthy based on a physical activity readiness questionnaire (PAR-Q) designed to identify individuals for whom PA might be inappropriate. These characteristics were selected to be controlled through the BYU Lifestyle study design because smoking and obesity tend to have many health-related confounding effects.

For the 2007-2008 follow-up, all 275 original participants will be contacted and invited to participate. Because loss to follow-up is a concern of the proposed study, steps have been taken to minimize non-participation by reducing the number of laboratory procedures, decreasing the length of time in the laboratory, and offering an incentive. Each participant will receive a $100.00 check after completing all the required steps of the study.

Design

This study will use a prospective cohort design. The exposure will be physical activity volume (PAv), physical activity intensity (PAi), and cardiorespiratory fitness (CRF) indexed by VO_2max. The outcome will be bone mineral density (BMD) and osteoporosis. Potential confounding variables, including age, dietary intake, baseline BMD, menopause status, baseline body mass index (BMI), BMI change, maternal history of osteoporosis, and dietary intake, will be statistically controlled. The study will be reviewed by the university Institutional Review Board.

In 1998, data collection began for the BYU Lifestyle Project, a prospective cohort study. Data collection included a comprehensive evaluation of several lifestyle-related disease risks. The 18-month follow-up occurred in 2000 and the same data were collected on 250 of the women, but included BMD using a bone densitometer. Since BMD is an integral factor of the proposed study, the 2000 phase will become the baseline for this study. Data collection during 2007 and 2008 will serve as the 7-year follow-up.

Procedures

To begin the 7-year follow-up assessment, the research team will call each participant and set up an appointment. The caller will ask the participant to visit the research facility one time for approximately 90 minutes in a three-hour fasted state, except for fluids. After the call, an Eating Behavior Questionnaire, a Block food frequency questionnaire, and an informed consent will be mailed to the participant to be filled out before the appointment.

Upon arrival at the body composition laboratory, a researcher will take the completed Eating Behavior Questionnaire, Block food frequency questionnaire, and informed consent from the participant and answer any questions the subject may have. After the participant is comfortable with the informed consent, she will be asked to eliminate any bodily waste and change into a BYU issued swimsuit. After the participant finishes changing her clothes, she will be asked to complete a Physical Activity and Lifestyle Questionnaire. While the participant is working on the questionnaire, the researcher will peruse the other questionnaires for mistakes and unanswered questions.

Once the questionnaires are finished, the technician will position participant on the bone densitometer for hip and spine scans. Once the scans are complete, the participant will change back into her normal attire.

Instrumentation and Measurement Methods

The instrumentation and measurement methods will be subdivided based on the study variables: physical activity, cardiorespiratory fitness, bone mineral density at the hip and spine, age, BMI, and dietary intake. Each section will be described according to the methods employed at the baseline data collection. Physical activity, cardiorespiratory fitness, BMD, menopause status, BMI, and dietary intake were measured in 2000. Bone mineral density, menopause status, BMI, maternal history of osteoporosis, and dietary intake will be measured in 2007-2008.

Physical Activity

At baseline, the women wore Computer Science Application, Inc (CSA, now Actigraph, Pensacola, FL) model 7164 accelerometers to measure PA. Physical activity

will not be measured as part of the 2007-2008 follow-up because it is an exposure variable of this prospective cohort study.

CSA accelerometers are both valid and reliable in estimating physical activity in the field.[49, 50] In a study comparing four popular activity monitors, the CSA accelerometer was the most accurate during moderate intensity physical activities in field and laboratory settings.[49] Furthermore, CSA accelerometers were strongly correlated to oxygen consumption measures obtained from portable metabolic systems.[49, 50] CSA activity counts have been reported to be highly correlated to steady-state oxygen consumption and significantly correlated with relative VO_2, heart rate, and treadmill speed.[50, 51]Participants were instructed regarding how to wear the monitor and they were encouraged to wear it at all times, except when engaging in water activities. Participants carried the accelerometer in a small pouch on a nylon belt at the height of the umbilicus, over the left hip, and along the outer seam of a pair of pants. Participants were asked to wear the accelerometers for seven consecutive days to provide extended evaluation of habitual PA. If participants failed to wear the activity monitor for three hours or more during the week, they were required to wear the accelerometer for another seven consecutive days.

Accelerometers record movement continuously and, in a week's time, most individuals have activity count totals in the millions. To keep the data more manageable, participants' output will be divided by 1000. The PAv variable will be indexed using the sum of all the activity counts over the 7-day period.

According to the 1995 CDC and ACSM recommendation on PA, "intermittent bouts of physical activity, as short as 8 to 10 minutes, totaling 30 minutes or more on most days provide beneficial health and fitness effects."[52] Therefore, the activity counts will be divided into 10-minute epochs as minimum intermittent bouts to be consistent with the CDC and ACSM guidelines. This will result in 1008 epochs over the seven-day testing period.

Variations in accelerometer activity counts within a specific time period (e.g., 10 minutes) reflect differences in PAi.[39-41] Published general reference criteria for three PAi categories will be formed reflecting low, moderate, and high physical activity intensities based on these criteria. A low PAi category associated with being sedentary to walking slowly (< 3mph) is defined by activity counts ranging from 0 to 29,000.[40] A moderate PAi category associated with walking slowly to briskly (3 to 4 mph) is defined by the range of 30,000 to 50,000 counts.[40] Finally, a high PAi category associated with movement equivalent to fast walking to running (> 4 mph) delimited to activity counts greater than 50,000.[40]

The basis of the PAi categories will be nine or more epochs (i.e., at least 90 minutes) including the highest activity counts within a particular intensity range (low, moderate, and high) over the seven days. In order to fit in the high PAi category, a participant must have 50,000 counts per epoch or greater for at least nine epochs during the seven days. For the moderate PAi categorization, a participant must have at least ninety minutes of activity counts equal to or greater than 30,000 and fail to fit into the

high PAi category. A participant in the low PAi category must have failed to accumulate at least nine epochs with intensities greater than 30,000 counts per epoch.

For example, if a participant has nine moderately intense epochs and nine high intensity epochs, then she would be placed in the high PAi category because she met the minimum requirement of at least nine epochs with activity counts greater than 50,000. Furthermore, if a participant had eight high intensity epochs and seven moderate intensity epochs, then she would be placed in the moderate PAi category because she had at least nine epochs with 30,000 counts or greater. The participant did not have at least nine epochs with 50,000 counts or greater so she would not be the high PAi category.

Cardiorespiratory Fitness

Cardiorespiratory fitness will be indexed by VO_2max using a graded maximal treadmill test (GXT) at baseline. Since CRF is, also, an exposure of this study, it will not be measured during the 2007-2008 follow-up.

Prior to the GXT, participants answered a PAR-Q to reveal any contraindications of the test. The research team instructed the participants of the protocol and showed them how to use the one-way breathing apparatus with their nostrils occluded. To ensure the safety of the participants,, they performed the GXT following guidelines from the American College of Sports Medicine.[54] The researchers used a mass spectrometer, medical gas analyzer (Marquette, St Louis, MO) and Consentius O_2 Uptake System (Consentius Technologies, Salt Lake City, UT) computer interface software to quantify expired gas concentrations and ventilatory volumes. A member of the research team

calibrated the analyzer each day prior to testing utilizing standard gas of known concentrations and a 3-L calibration syringe (Hans Rudolph, Inc. MO).

The participants performed the GXT following the modified George protocol for the measurement of VO_2max.[48] George reported that this protocol has stable results across subgroups with high *r* values (≥ 0.95) and low SEE (< 2.0) generated by a double, cross validation procedure.[48] He also reported an intraclass test-retest reliability of the modified George Protocol to be 0.99.[48]

Following the modified George protocol, participants walked on a motor-driven Quinton Model 65 treadmill (Seattle, WA) at a 5% grade at a self-selected, brisk pace during the first 3-min stage of the GXT. At the beginning of the second 3-min stage, participants selected a comfortable jogging pace after the technician decreased the grade of the treadmill to zero degrees. Following the second 3-minute stage, the technician increased the treadmill grade by 1.5% every minute until the participant could not continue.

A Polar electronic monitoring system (Polar Inc., Westbury, NY) measured the participant's heart rates. Participants reported their perceived exertion by selecting a rating of perceived exertion (RPE) from a scale of 6 to 20 based on the Borg scale.[48] The examiner recorded the heart rates and RPE after the two 3-min stages and at the end of each subsequent minute. A rolling estimation of the four highest sequential VO_2 measures, which were reported every fifteen seconds, was used to determine the participant's VO_2max.

The maximal GXT was determined when three of the four following criteria were accomplished: (1) participant's heart rate reached within 15 beats of the predicted maximal heart rate (220 – age), (2) VO_2 did not increase after a sustained increase in treadmill grade, (3) participant's RPE reached 19 or 20, and (4) a maximal Respiratory Exchange Ratio (RER) of 1.1 or greater was achieved. Participants who failed to achieve a maximal test were retested approximately one week later.

Bone Mineral Density

Bone mineral density of the hip and spine was measured using dual energy x-ray absorptiometry (DEXA) at baseline. A Hologic QDR 4500 W (Waltham, MA) bone densitometer was employed to measure BMD at two locations, the hip and lumbar spine (i.e. L1-L4). DEXA is considered to be a reliable and valid procedure for measuring bone mass and density as a surrogate of fracture risk.[8, 18, 55] The 7-year follow-up will employ the same procedure using DEXA to measure BMD at the hip and spine for the outcome variables of this proposed study.

On each day of testing, the technician calibrated the DEXA before any scans were performed. The DEXA scan arm emits laser light cross-hairs to facilitate positioning the x-ray arm and improving accuracy of the scan. The technician used the QDR 11.2 scan software (Hologic, Waltham MA) to analyze the participant's BMD.

For the hip scan, the technician positioned the laser cross-hairs of the scan so that they bisected the left thigh at the level of the pubic bone. The technician internally rotated the participants' left leg, slightly abducted the leg, and strapped it to a positioning aide.

This position minimizes the view of the lesser trochanter and the femoral shaft on the scan image.

For the spine scan, the technician placed a rectangular foam box under the legs of the participant to flatten the lordosis of the spine. The cross-hairs of the laser were aligned to bisect the body vertically, one inch below the highest level of the iliac crest. This positioning allows for scanning L1-L4 vertebral bodies and increases accuracy of the spine scan.

To diagnose osteoporosis, the BMD values of the hip and spine will be compared to the standard mean peak bone mass of a healthy, young reference population. The World Health Organization diagnostic criterion for osteoporosis will be used and is defined as a T score of less than or equal to -2.5.[1, 18, 19, 74] Osteopenia, or low bone mass, is defined as a T score between -1.0 and -2.5, and normal bone density is a T score of at least -1.0.[1, 19, 74]

Menopause Status

At baseline, menopause status of the participants was divided into three categories: premenopausal, perimenopausal, and postmenopausal using a series of six questions. The menopause questions focused on the amount of time since the participants' last menstrual cycle, the regularity of menstrual cycles, and the presence of common symptoms associated with menopause. The same series of six questions will be posed in the 2007-2008 for the follow-up.

Body Mass Index

Weight at baseline was measured on an electronic scale, model Profit/UC-321 (Life Source, Milpitas, CA) to the nearest 0.05 kg. Height was measured to the nearest 0.001 meter using a wall-mounted stadiometer, model 439 (Cardinal Scale Manufacturing, Webb City, MO). Body mass index (BMI) was calculated using the standard formula of mass in kilograms divided by height in meters squared. Follow-up BMI will be calculated in 2007-2008 in the same manner as in 2000 with the new weight and height data.

Dietary Intake

Research shows that dietary intake of calcium, protein, magnesium, phosphorus, vitamin D, potassium, fluoride, manganese, copper, boron, iron, zinc, vitamin A, vitamin K, vitamin C, and the B vitamins are significant predictors of BMD in women.[4, 18, 64] Thus, dietary intake of calcium, protein, magnesium, phosphorus, vitamin D, potassium, fluoride, manganese, copper, boron, iron, zinc, vitamin A, vitamin K, vitamin C, and the B vitamins were assessed using the Block food frequency questionnaire (FFQ) at baseline. The Block FFQ assesses the portion size and frequency of consumption of over 100 different foods and drinks and is considered a valid and reliable measure of food and beverage intake.[60-63] Dietary intake will be assessed again at the seven-year follow-up using the Block FFQ.

Maternal History of Osteoporosis

The participants' self-reported maternal history of osteoporosis will be assessed using a question that asks if the participants' mother was diagnosed with osteoporosis

before the age of 65 by a physician. This variable was not measured at baseline, but will be assessed at the 2007-2008 follow-up.

Statistical Analysis

A power analysis was conducted using the PASS 6.0 statistical software to determine the number of participants needed to detect an effect size of 0.25 with power at 0.80 and alpha at the 0.05 level. Results showed that 200 subjects would be sufficient.

All statistical analyses will be computed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, 2003). Descriptive data, including frequencies, means, and standard deviations, will be generated for each variable. Changes in BMD and BMI will be calculated by subtracting baseline values from follow-up values taken seven years later. Participants with T scores of less than or equal to -2.5 for the hip or spine will be considered osteoporotic for that body region.

Participants will be divided into quartiles based on their CRF levels, and the middle-two quartiles will be collapsed to form three total CRF groups. Women will also be divided into quartiles based on their physical activity volume levels (PAv). Like before, the middle-two quartiles will be combined forming three total PAv groups. Lastly, participants will also be divided into three groups based on their physical activity intensity (PAi) levels, as discussed in the methods section.

Chi-square analyses will be conducted to determine the associations between PAv, PAi, and CRF, treated as categorical variables, and the outcome variables. Relative risk will be calculated using the PROC GENMODE command in SAS by comparing incidence rates over the seven year study. Specifically, risk of BMD loss at the hip and

the spine will be determined by comparing the incidence rate of bone loss among women with high levels of PAv to those with typical levels and again to those with low levels of PAv. Similarly, risk of bone loss will be assessed by comparing the incidence rate among women with high levels of PAi to their counterparts. Additionally, risk of BMD loss will be determined by comparing the incidence rate among women with high levels of CRF to those with normal levels and again to those with low levels of CRF. The same types of calculations of relative risk will be computed to determine risk of developing osteoporosis at the hip or spine among participants with differing levels of PAv, PAi, and CRF. Statistical significance will be based on the 0.05 level. The relative risk calculations will be adjusted for the potential confounding effects of age, time between the assessments, dietary intake, baseline BMI, changes in BMI, baseline BMD, maternal history of osteoporosis, and menopause status, treated individually and in combination, using a modified Poisson regression approach with robust error variance, as explained by Zou.[66]

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Appendix B

Addendum

ADDENDUM

According to the approved prospectus, the purpose of this prospective cohort study was to assess the extent to which objectively measured PAv, PAi, and CRF influence the likelihood of significant BMD gains in the hip and spine and the risk of osteoporosis development in middle-age women over a six-year period. A secondary purpose was to ascertain the extent to which age, dietary intake, baseline mass, changes in mass, baseline BMD, changes in perceived fitness, maternal history of osteoporosis, and menopause status influence these relationships over time.

All of the purposes of the proposed research were studied. However, no significant relationships were observed between PAi and hip BMD changes, nor CRF and hip BMD changes. There were also no significant relationships observed between PAv and spine BMD changes, PAi and spine BMD changes, nor CRF and spine BMD changes. Each of these associations was evaluated, however, because the vast majority of the relationships were not significant, and because there were numerous relationships evaluated, it was not reasonable to report them all in an article for publication.

None of the relationships between PAv, PAi, nor CRF and spine BMD were statistically significant. Hence, results associated with these findings were placed in tables which were included in this Addendum.

Osteoporosis and osteopenia

To diagnose osteoporosis, BMD values of the hip and spine were compared to the standard mean peak bone mass of a healthy, young reference population. The World Health Organization diagnostic criterion for osteoporosis was used and has been defined as a hip BMD of less than or equal to 0.637 mg/cm^2 for the Hologic DEXA.[1, 18, 19]

Osteopenia, or low bone mass, has been defined as a hip BMD between 0.820 mg/cm^2 and 0.637 mg/cm^2, and normal bone density as a hip BMD greater than 0.820 mg/cm^2.[1, 18]

Participants with a hip BMD between 0.820 mg/cm^2 and 0.637 mg/cm^2 were diagnosed to have osteopenia and participants with a hip BMD of less than or equal to 0.637 mg/cm^2 were considered osteoporotic. The same types of calculations of relative risk were computed to determine risk of developing osteoporosis and osteopenia at the hip or spine among participants with differing levels of PAv, PAi, and CRF.

A total of 44 participants had hip BMD scores less than 0.820 mg/cm^2 at baseline. Only 17 of the participants developed osteopenia between the baseline and follow-up. This number of participants was insufficient to separate into BMD change groups and analyze for relative risk.

ADDENDUM TABLES

Addendum Table 1 Likelihood of significant hip BMD gains ($\geq 75^{th}$ percentile) in High and Moderate PAi compared to Low PAi women at baseline over 6 years

	Outcome: Increased BMD ($\geq 75^{th}$ percentile)					
	High-Moderate vs Low PAi		Moderate vs Low PAi		High vs Low PAi	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	0.98	0.56-1.72	1.16	0.62-2.18	.	.
Age	0.98	0.56-1.72	1.17	0.63-2.21	.	.
CRF	1.18	0.64-2.17	1.25	0.66-2.37	.	.
Baseline hip BMD	0.98	0.56-1.72	1.17	0.62-2.20	.	.
Baseline mass	0.99	0.56-1.75	1.15	0.61-2.18	.	.
Mass change	0.99	0.56-1.75	1.18	0.63-2.23	.	.
Time in the study	0.81	0.47-1.37	0.98	0.57-1.66	.	.
Menopause status	0.92	0.54-1.56	1.06	0.58-1.93	.	.
Calcium intake	0.91	0.52-1.59	1.04	0.55-1.97	.	.
Vitamin D intake	0.98	0.56-1.72	1.16	0.62-2.17	.	.
Perceived fitness Change	0.98	0.56-1.72	1.14	0.62-2.09	.	.
All of the above	0.76	0.42-1.37	0.81	0.45-1.46	.	.

RR=relative risk. Low PAi was the reference category.

95% CI=95% confidence interval.

Low PAi included women with 15 intervals of activity counts <30,000 per epoch (reference group: n=200), Moderate PAi included women with 15 epochs of activity counts between 30,000 to 50,000 counts per epoch (n=27), and High PAi included women with 15 epochs of >50,000 counts per epoch (n=17). High vs. low PAi could not be performed because the high PAi group had only 3 participants who had favorable changes in BMD. None of the comparisons were statistically significant.

Addendum Table 2 Likelihood of significant hip BMD gains (≥ 75th percentile) in High and Moderate CRF compared to Low CRF women at baseline over 6 years

	Outcome: Increased BMD (≥ 75th percentile)					
	High-Moderate vs Low CRF		Moderate vs Low CRF		High vs Low CRF	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	0.66	0.37-1.16	1.01	0.62-1.66	0.67	0.35-1.27
Age	0.64	0.36-1.12	1.00	0.61-1.64	0.58	0.30-1.13
PAv	0.53	0.28-1.00	0.83	0.50-1.40	0.53	0.22-1.29
Baseline hip BMD	0.66	0.38-1.16	1.01	0.62-1.65	0.66	0.35-1.26
Baseline mass	0.67	0.37-1.20	1.00	0.61-1.65	0.75	0.36-1.56
Mass change	0.66	0.37-1.15	1.04	0.63-1.69	0.67	0.35-1.27
Time in the study	0.68	0.40-1.20	0.98	0.61-1.57	0.68	0.36-1.28
Menopause status	0.63	0.36-1.10	0.93	0.58-1.50	0.59	0.31-1.14
Calcium intake	0.67	0.39-1.17	1.05	0.65-1.69	0.70	0.37-1.33
Vitamin D intake	0.67	0.38-1.19	1.02	0.62-1.66	0.70	0.36-1.34
Perceived fitness change	0.71	0.40-1.27	1.02	0.62-1.69	0.76	0.38-1.53
All of the above	0.61	0.32-1.17	0.80	0.47-1.38	0.60	0.22-1.57

RR=relative risk. Low CRF was used as the reference category.

95% CI=95% confidence interval.

Low CRF included women in the lowest quartile (reference group: n=65). Moderate CRF included women in the middle-two quartiles (n=114). High CRF included women in the highest quartile (n=65). None of the comparisons were statistically significant.

Addendum Table 3 Likelihood of significant spine BMD gains ($\geq 75^{th}$ percentile) in High and Moderate PAv compared to Low PAv women at baseline over 6 years

	Outcome: Increased BMD ($\geq 75^{th}$ percentile)					
	High-Moderate vs Low PAv		Moderate vs Low PAv		High vs Low PAv	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	1.04	0.63-1.72	1.10	0.65-1.87	0.92	0.49-1.74
Age	0.97	0.59-1.59	1.03	0.61-1.75	0.80	0.42-1.51
CRF	1.08	0.63-1.83	1.13	0.66-1.94	0.92	0.42-2.01
Baseline spine BMD	1.04	0.62-1.72	1.09	0.64-1.85	0.92	0.49-1.74
Baseline mass	1.04	0.62-1.72	1.08	0.64-1.83	0.89	0.47-1.67
Mass change	1.03	0.59-1.69	1.07	0.64-1.80	0.96	0.51-1.81
Time in the study	1.03	0.62-1.71	1.09	0.64-1.87	0.86	0.44-1.66
Menopause status	0.91	0.56-1.48	1.00	0.60-1.66	.	.
Calcium intake	1.05	0.63-1.73	1.10	0.65-1.87	0.94	0.50-1.76
Vitamin D intake	1.06	0.64-1.74	1.11	0.66-1.87	0.98	0.52-1.85
Perceived fitness change	1.07	0.65-1.77	1.12	0.67-1.91	0.97	0.51-1.82
All of the above	1.05	0.61-1.81	1.02	0.58-1.77	.	.

RR=relative risk. Low PAv was used as the reference category.

95% CI=95% confidence interval.

. = Insufficient number of participants for the covariates.

Low PAv included women in the lowest quartile (reference group: n=61). Moderate PAv included women in the middle-two quartiles (n=118). High PAv included women in the highest quartile (n=62). None of the comparisons were statistically significant.

Addendum Table 4 Likelihood of significant spine BMD gains (≥ 75th percentile) in High and Moderate PAi compared to Low PAi women at baseline over 6 years

	Outcome: Increased BMD (≥ 75th percentile)					
	High-Moderate vs Low PAi		Moderate vs Low PAi		High vs Low PAi	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	0.58	0.28-1.19	.	.	.	.
Age	0.58	0.29-1.18	.	.	.	.
CRF	0.56	0.27-1.17	.	.	.	.
Baseline spine BMD	0.58	0.28-1.18	.	.	.	.
Baseline mass	0.59	0.29-1.20	.	.	.	.
Mass change	0.60	0.29-1.23	.	.	.	.
Time in the study	0.55	0.27-1.16	.	.	.	.
Menopause status	0.51	0.26-1.04	.	.	.	.
Calcium intake	0.59	0.29-1.20	.	.	.	.
Vitamin D intake	0.58	0.28-1.18	.	.	.	.
Perceived fitness change	0.58	0.28-1.20	.	.	.	.
All of the above	0.46	0.21-1.02	.	.	.	.

RR=relative risk. Low PAi was used as the reference group.

95% CI=95% confidence interval.

. = Insufficient number of participants for the covariates.

Low PAi included women with 15 epochs of activity counts <30,000 per epoch (reference group: n=197), Moderate PAi included women with 15 epochs of activity counts between 30,000 to 50,000 counts per epoch (n=27), and High PAi included women with 15 epochs of >50,000 counts per epoch (n=17). Moderate vs. low and high vs. low PAi could not be performed because of sufficient numbers of participants in the moderate PAi and the high PAi groups with favorable changes in BMD. None of the comparisons were statistically significant.

Addendum Table 5 Likelihood of significant spine BMD gains ($\geq 75^{th}$ percentile) in High and Moderate CRF compared to Low CRF women at baseline over 6 years

	Outcome: Increased BMD ($\geq 75^{th}$ percentile)					
	High-Moderate vs Low CRF		Moderate vs Low CRF		High vs Low CRF	
Variable Controlled	RR	95% CI	RR	95% CI	RR	95% CI
None	1.11	0.67-1.84	1.18	0.69-2.01	0.98	0.53-1.84
Age	1.04	0.63-1.72	1.16	0.68-1.97	0.77	0.42-1.41
PAv	1.18	0.68-2.05	1.25	0.71-2.22	0.81	0.37-1.76
Baseline spine BMD	1.11	0.67-1.85	1.19	0.70-2.02	0.99	0.53-1.85
Baseline mass	1.16	0.69-1.94	1.21	0.71-2.07	1.10	0.55-2.17
Mass change	1.16	0.69-1.96	1.27	0.73-2.21	0.99	0.53-1.89
Time in the study	1.11	0.66-1.84	1.17	0.69-2.00	0.99	0.53-1.85
Menopause status	0.98	0.60-1.61	1.05	0.63-1.76	0.82	0.44-1.54
Calcium intake	1.10	0.66-1.83	1.17	0.68-1.99	0.98	0.52-1.84
Vitamin D intake	1.08	0.65-1.79	1.16	0.68-1.97	0.95	0.50-1.78
Perceived fitness change	1.18	0.72-1.96	1.29	0.76-2.17	0.98	0.54-1.78
All of the above	1.29	0.72-2.30	1.51	0.79-2.87	0.59	0.26-1.32

RR=relative risk. Low CRF was used as the reference category.

95% CI=95% confidence interval.

Low CRF included women in the lowest quartile (reference group: n=65). Moderate CRF included women in the middle-two quartiles (n=114). High CRF included women in the highest quartile (n=65). None of the comparisons were statistically significant