

BONE MINERAL DENSITY IN COLLEGE FEMALE ATHLETES AND NON-ATHLETES:

A THREE YEAR STUDY

by

CLAIRE VICTORIA MAUST

(Under the Direction of Richard D. Lewis)

ABSTRACT

The purpose of this study was to determine how sports participation during three college years affects areal bone mineral density (aBMD) in female artistic gymnasts (GYM; N=37), cross-country runners (RUN; N=28), and non-athlete controls (CON; N=69). Body composition and total hip, femoral neck, trochanter, and lumbar spine bone measurements of GYM, RUN, and CON were determined using dual X-ray absorptiometry. Using SAS software, data were analyzed using linear models and fixed effects were performed using ordinary least squares linear regression. Total hip and femoral neck aBMD significantly declined in CON, but not in GYM or RUN ($p<0.0001$). Trochanter aBMD declined significantly ($p<0.0001$) in all groups. GYM but not RUN or CON lumbar spine aBMD increased significantly ($p<0.05$). These results suggest that participation in weight-bearing sports during the collegiate years has a beneficial and/or protective effect on total hip, femoral neck and lumbar spine aBMD, but not necessarily the trochanter.

INDEX WORDS: Gymnasts, Runners, Bone mass, Young adults

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CLAIRE VICTORIA MAUST

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CLAIRE VICTORIA MAUST

Major Professor:	Richard D. Lewis
Committee:	Clifton A. Baile Alex Kojo Anderson

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION

Osteoporosis affects approximately 200 million people worldwide and osteoporosis-related fractures are estimated to occur during the remaining lifetime of 60% of women over age 50 years (1, 2). Often considered a disease of the elderly, the pathogenesis of osteoporosis potentially lies in childhood and adolescence since, by age 12-18 years, approximately 80-90% of total adult bone mass is accrued (3-6). Given that low bone mass reduces bone strength and leads to fractures, maximizing bone mass and strength during growth to achieve optimal peak bone mass (PBM) is important to decrease fracture risk later in life (7, 8). Two strategies recommended to reduce the risk of osteoporotic fractures are to increase PBM during growth and to reduce the rate of bone loss that occurs in adulthood. It has been estimated that a 10% increase in PBM could delay the development of osteoporosis by 13 years (9) and reduce fracture risk by 50% (10). Even small areal bone mineral density (aBMD) gains during the period close to PBM attainment could have a profound effect on the prevention of osteoporotic fractures later in life (11).

Peak bone mass is defined as the “the full genetic potential for bone strength” (12), “the amount of bony tissue present at the end of skeletal maturation” (13) or “the highest level of bone mass achieved as a result of normal growth” (11). The ‘definition’ of PBM is as ambiguous as its timing. The majority of cross-sectional and prospective studies suggest that PBM, depending on the skeletal site, is likely reached by the second or third decade of life followed by a 1% loss per year thereafter, yet the exact timing of PBM attainment remains

unclear (11, 14-16). The lumbar spine aBMD peaks in the mid-20s (13, 17) whereas the total hip and femoral neck peak earlier, around age 16-18 years (3, 17). While the majority (approximately 90%) of total adult bone mass is achieved by age 18 years (13, 16), up to 12.5% increases in total body aBMD have been observed over the third decade (18), suggesting that strategies to maximize bone mass during the young adult years may still be beneficial. Several lifestyle factors that may influence bone mineral accrual during growth include diet/nutrition, hormones, oral contraceptives, menarchal status, menarchal age, and physical activity. “Of all the modifiable lifestyle factors that influence the skeleton...it is exercise during growth that has the potential to...reduce the public health burden of fractures” (19).

The beneficial role of physical activity and weight-bearing exercise on bone gains has been clearly documented in cross-sectional (20-22) and longitudinal (23, 24) studies of growing children. Observational studies of physically active children and adolescents also provide evidence of greater aBMD gains, 2-4% in some cases, over their less physically active peers (25-27). Several randomized controlled physical activity and jumping intervention trials on growing children show greater bone gains in jumpers and exercisers than controls (28-31). Organized sports groups (i.e., gymnastics, cross-country running, swimming, etc.) are often used in physical activity and bone studies since the bone-loading maneuvers performed during the training regimens of sports are distinct and similar within each sport group. Cross-sectional studies of child (21) and adolescent (32) gymnasts show that the impact-loading forces incurred during performance of gymnastic maneuvers are beneficial to bone mass gains (23, 24). Artistic gymnastics employs maneuvers shown to generate high impact forces [up to 10-12 times body weight (33)] and is a sport known to augment bone mass development (24, 32, 34). Furthermore, studies suggest that the benefits of physical activity on bone gained during childhood and

adolescence are greater with high impact activities and are maintained into young adulthood (35, 36).

Since bone gains are less dramatic in the young adult years than during puberty, the assumption is made that there is not much that can be done to gain bone mass during the college years. Below are physical activity and bone studies conducted to date in young adults.

Cross-sectional studies of young adult athletes participating in various sports show that athletes participating in sports with more high impact and weight-bearing loads have increased bone mass and strength (37-41). For example, weight-bearing activities like volleyball, tennis, running, and power lifting benefit bone mass development (37, 42), whereas non-weight-bearing activities (i.e., swimming and cycling) consistently show no significant difference in aBMD vs. controls (43-45). Collegiate artistic gymnasts are known to have significantly higher aBMD than non-athlete controls at the total body, total hip, femoral neck, and lumbar spine (46). Over an 8 month prospective study of collegiate gymnasts, runners and controls, lumbar spine aBMD gains of the gymnasts were significantly greater than both runners and controls ($2.8 \pm 2.4\%$ vs. $-0.2 \pm 2.0\%$ vs. $0.7 \pm 1.3\%$), respectively (45). At the femoral neck, runners experienced a significant loss of aBMD while gymnasts experienced a significant gain over 8 months ($p < 0.05$) (45). Significant changes in aBMD over 8 months during the college years suggest that the potential for additional bone mineralization still exists. A study assessing the change in bone throughout all four of the college years in 164 highly physically active Caucasian females students found significant increases in all bone sites except the trochanter (47). This study reported significant ($p < 0.0001$) increases in aBMD at the hip (2.26%), lumbar spine (3.27%), and total body BMC (5.25%), and a non-significant ($p > 0.05$) decline in trochanter aBMD (-0.6%) over 3.6 years (47).

However, it is not known if the students' activity led to these changes because there was no control group (47).

To our knowledge, the two aforementioned prospective studies are the only studies focusing on the change in aBMD in physically active females during the college years. In order to determine the potential for physical activity to modify bone in young adulthood specifically, the current study was performed to assess changes in aBMD at the total hip, femoral neck, trochanter, and lumbar spine over 36 months in collegiate artistic gymnasts, cross-country runners, and healthy non-athlete (i.e., control) females. We hypothesized that aBMD of the lumbar spine, but not the hip, increases in college-aged female collegiate artistic gymnasts, cross-country runners, and non-athlete controls over 36 months with gymnasts experiencing the greatest increase.

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CHAPTER 2

REVIEW OF THE LITERATURE

In order to provide greater insight into the current study, the following chapter will present background information on bone biology, bone development during growth, the theory of peak bone mass (PBM), measurements of bone, and factors affecting bone health. Although genetic factors have the greatest influence on bone, modifiable factors significantly affect the attainment of PBM and bone strength. Specific factors that will be discussed include dietary calcium and vitamin D, supplement use, age at menarche, menarchal status, oral contraceptive use, and physical activity.

Bone Biology

Anatomy

Bone is a dynamic tissue essential to maintaining mineral homeostasis (1). The skeleton is also vital to the body for mechanical support, protection of body organs, and for functioning in movement (2). There are two types of bone: cortical (compact) and trabecular (cancellous) bone. Cortical bone, accounting for 80% of skeletal mass, is the dense calcified tissue that makes up the outer portion of most bones and the shaft of long bones (3). The hard, rigid cortical bone tissue provides the skeleton with compression strength and mechanical structure (4). Trabecular bone is predominately located in the axial skeleton and in the ends of long bones. Spongy in appearance, trabecular bone consists of calcified honeycomb-like structures known as trabeculae that increase the surface area of the bone. The increased surface area allows for more contact

with blood vessels, connective tissue, and bone marrow, creating a more metabolically active environment to maintain calcium homeostasis (3, 5-7).

Since trabecular bone is more metabolically active than cortical bone, bone sites containing significant quantities of trabeculae “turn over” more rapidly than bone sites with more cortical bone (8). Bone turnover will be discussed in more detail in the “bone modeling and remodeling” section below. Therefore, significant changes in bone mineral content (BMC) and areal bone mineral density (aBMD) at the lumbar spine, which is composed of 66% trabecular bone (8), can be observed in a few months; whereas as changes in the femoral neck, which has about 25 % cortical bone (9), may take years (10, 11). With more rapid bone turnover, trabecular bone loss begins earlier, as in young adulthood, whereas cortical bone loss does not occur until later, as during menopause (12, 13). Moreover, skeletal sites with the most trabecular bone are at the greatest risk for fracture.

Bone Modeling and Remodeling

During growth, bone lengthens, consolidates, and changes shape in response to the various aforementioned stimuli that initiate bone resorption and formation or “bone turnover.” Whereas bone modeling occurs from childhood until early adolescence and ultimately results in bone growth (14, 15), bone remodeling occurs throughout the entire lifecycle and is important for mineral homeostasis and bone repair (figure 2.1) (6, 16). Skeletal cells within the bone that play crucial roles in bone turnover include osteoblasts (mononucleated cells responsible for bone formation), osteoclasts (multinucleated cells that resorb bone), bone lining cells (inactive, flattened osteoblasts), and osteocytes (mature osteoblasts calcified within the bone matrix) (2, 17). Bone lining cells cover the surface of the bone, receive signals from osteocytes and regulate the deposition and resorption of calcium by mediating the actions of osteoblasts and osteoclasts

(18). Osteocytes are mechanoreceptors that signal bone-lining cells to stimulate the resorption and formation process in response to fractures. Other stimuli that initiate resorption and/or formation processes include estrogen deficiency, low serum calcium levels, diseases, medications, and/or the type of loading forces on bones (6).

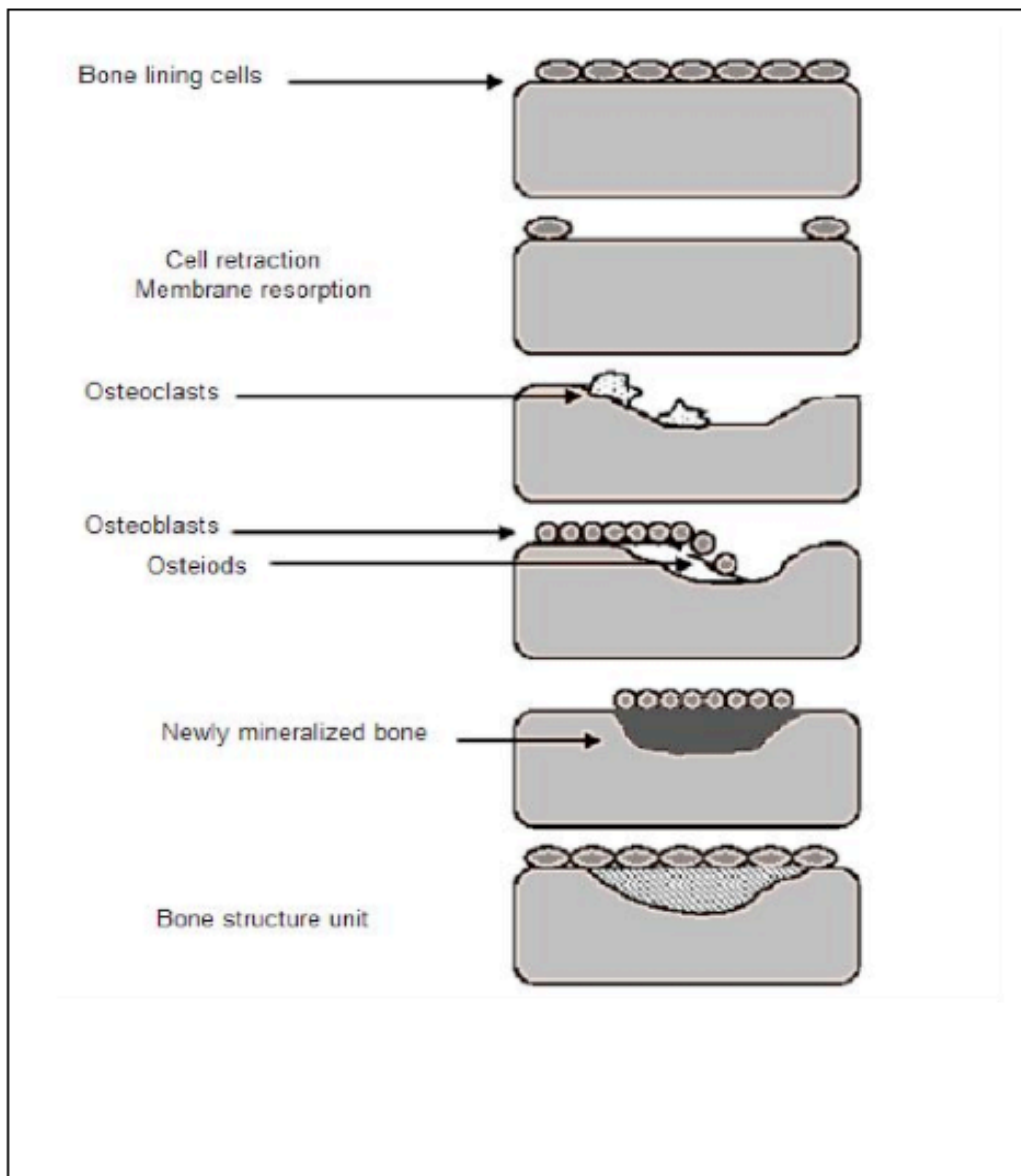


Figure 2.1. Bone remodeling process. Adapted from Compston et al (16).

During bone modeling, osteoclasts and osteoblasts resorb and form bone, respectively, at different sites on bone, in an uncoupled fashion, such that formation exceeds resorption.

Conversely, during bone remodeling osteoclast and osteoblast activities are coupled throughout most of young adulthood and occur at the same site on bone. In normal conditions (i.e., in healthy young adults), bone mass is conserved during this bone remodeling process (19); however, in individuals with low estrogen levels (e.g., in post-menopausal women), increased bone resorption and decreased bone formation leads ultimately to bone loss (14, 20, 21). Since approximately half of all women over 50 years of age will have an osteoporotic fracture during their remaining lifetime

(22), even small gains in areal bone mineral density (aBMD) in the young adult years could have a profound effect on preventing osteoporotic fractures later in life (figure 2.2) (23, 24).

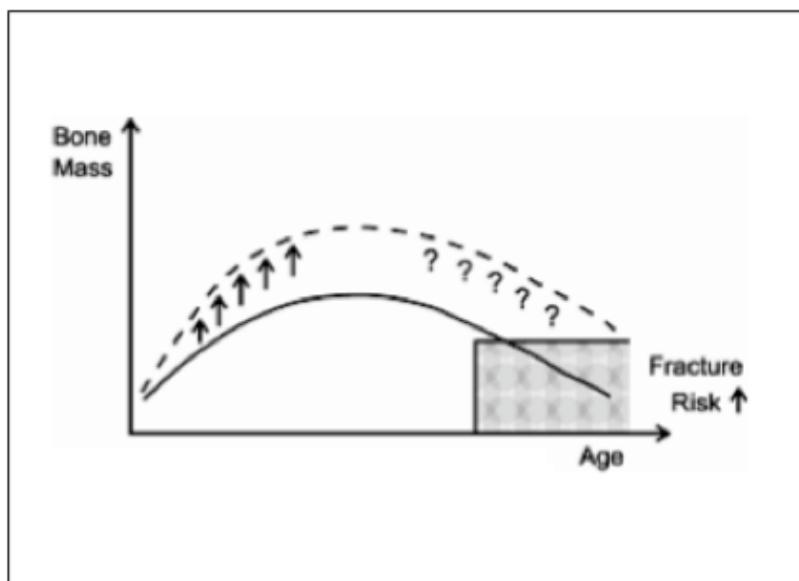


Figure 2.2. Peak bone mass. Adapted from Schonau et al, 2004 (23)

Bone Development During Growth

Bone grows at various rates throughout life. The first 20 years exhibit a period of rapid growth (about 90% of total potential bone growth), followed by years of maintenance and subsequent bone loss typically observed throughout aging and specifically during menopause

(25). During a 3-4 year window in early to mid-puberty, termed the “critical years” of bone formation, the maximal rate of bone mineral accrual takes place. Skeletal muscle also develops prior to and proportionally to bone mass development as hypothesized in the mechanostat theory: increased muscle mass or maximal muscle force during growth increases bone mass, size and strength comparably (26, 27). Peak height velocity, the maximal rate of height growth, precedes peak bone mineral accrual, creating a time when bones are long but thin because the osteoids have not yet mineralized (28). Shortly after maximum height is reached, epiphyseal plates, at the end of bones, completely ossify and bones stop growing in length but can continue to grow appositionally if conditions are favorable (29). The point at which bone modeling ceases and bones reach maximal mass is unclear.

Peak Bone Mass

The ‘definition’ of peak bone mass (PBM) is as ambiguous as its timing. Researchers define PBM as “the full genetic potential for bone strength” (30), “the amount of bony tissue present at the end of skeletal maturation” (31) or “the highest level of bone mass achieved as a result of normal growth” (23). The majority of cross-sectional and prospective studies suggest that PBM, depending on the skeletal site, is likely reached by the second or third decade of life followed by a 1% loss per year thereafter, yet the exact timing of PBM attainment remains unclear (23, 32-34).

While cross-sectional investigations have attempted to pinpoint when PBM occurs at various skeletal sites, results vary among the populations studied. Matkovic et al (23) found that bones finish growing in length around age 16 years, as evidenced by maximal height gains. This study also reported that hip aBMD reached a maximum at age 17 years and did not see any significant changes in total body bone mineral content (BMC) and aBMD past age 18 years (23).

Walsh et al (23, 31) reported that lumbar spine PBM occurred at 18 years of age. However, Matkovic et al (23) reported 7% lumbar spine BMC increases in males and females between ages 19 to 50 years due to the broadening of the lumbar vertebrae bodies, and a study of 225 women aged 18-52 years reported 5% lumbar spine aBMD increases from the early 20s to the mid-30s (33).

Much like the previously mentioned cross-sectional studies, prospective studies also show a range of PBM values. In a prospective study of 156 healthy college-age female students aged 19-27, the median bone gains were 5.9% lumbar spine BMC, 6.8% lumbar spine aBMD, and 12.5% total body aBMD ($p < 0.0001$ for all) over the third decade (35). In this study, total body PBM was estimated to occur between 28.3 – 29.5 years of age, suggesting that bone mass continues to increase beyond the cessation of linear bone growth (35). The FELS Longitudinal Study found that females attained PBM earlier than males and that peak BMC and aBMD were reached at age 20 and 25 years, respectively (36). A study of 330 healthy 7-47 year old females also reported PBM attainment around 20 years of age (37). Other prospective studies suggest that healthy adolescent females may attain lumbar spine and total body PBM as young as 15-16 years of age and 14 years at the femoral neck (38, 39). To date, cross-sectional and prospective data shed some light on PBM attainment but are inconclusive regarding the exact timing of PBM at key fracture sites within the skeleton.

Measurements of Bone

To measure changes in aBMD, dual energy X-ray absorptiometry (DXA) is most commonly used since it is considered the “gold standard” for measuring aBMD in order to estimate fracture risk. Areal BMD is one of the primary predictors of fracture risk and osteoporosis assessments (40, 41). DXA provides a measure of bone area (cm^2), BMC (g), and

aBMD (g/cm^2) but does not give a true volumetric measure of BMD (g/cm^3) (42). Whereas bone mass (g) refers to the amount of mineral in bone, BMC (g) refers to bone mass within a designated region of interest (43). Areal BMD provides a two-dimensional view of bone geometry taking into account BMC and the average bone area (cm^2) (44). In comparison to true volumetric BMD (g/cm^3), aBMD is limited when interpreting bone strength. For example, volumetric BMD is relative to the outer bone volume regardless of bone size, but two-dimensional aBMD is affected by bone size (24). Though DXA measurements are indeed influenced by bone size (45), bone data derived from DXA predict fracture risk and are often used as reliable measures in research and clinical settings (42, 46). The study in chapter 3 will focus on aBMD measurements of the femoral neck, trochanter, total hip, and lumbar spine.

Analyses of aBMD using DXA compare an individual's aBMD to a reference aBMD, expressing the difference in standard deviation units as a Z-score or a T-score. Z-scores compare an individual's aBMD to the mean of age-matched aBMD data for a particular site. T-score values compare the patient's aBMD with the young adult-normal mean aBMD, based on data from cross-sectional studies of healthy 20- to 30-year-old sex-matched controls who have presumably reached PBM (24, 42). Hip T-scores are based on data from 18- to 20-year-olds and spine T-scores use data from 30-year-olds based on a consensus from existing PBM research. However, the exact timing of PBM is still unclear, and more prospective studies are needed to alleviate the controversy (23, 47). To analyze lumbar spine aBMD in college-aged or younger subjects, the Z-score is used versus the T-score. Use of the T-score is not an accurate comparison, as it is based on the mean aBMD of older subjects who have had more time for bone mineralization (24).

Factors Affecting Bone Health

Approximately 60-80% of PBM is determined by unmodifiable genetic factors, while 20-40% of possible PBM attainment is determined by environmental and lifestyle factors (48, 49). The following section describes such factors as diet (mainly supplement use, calcium, and vitamin D), hormones, menarchal status, and physical activity.

Diet

The college years can be a dynamic transition for students as they often represent the first time away from home, when individual dietary habits are established and food choices are abundant (50). College students typically consume a diet lacking in fruits, vegetables, and dairy products and high in fat, sodium and sugar (50, 51). Based on their weight and activity level, competitive college athletes are also at risk for inadequate nutrient intake, given their specialized nutrient requirements. Artistic gymnasts and cross-country runners are the focus of chapter 3.

According to a retrospective study of college gymnasts (n=26) and non-athlete controls (n=26), self-reported caloric intakes of both groups were found to meet only 63% and 79%, respectively, of the Recommended Daily Allowances (RDA) for kilocalories (kcal) for their age group, 2,200 kcal/day (52, 53). Gymnasts, particularly, consumed significantly lower kcal than controls ($P < 0.05$) and expended significantly ($p < 0.0001$) more energy than controls in a cross-sectional study of 26 collegiate gymnasts and 26 age-matched controls (53). A study of 91 female competitive runners aged 18-26 reported kcal intakes of approximately 2230 ± 133 kcal/day (54) whereas observations of 26 young adult female competitive runners reported total energy intakes of 1513 ± 538 kcal/day (55). Aforementioned observational data suggest that gymnasts and runners may not consume adequate calories and that, on average, college students may or may not meet energy and/or nutrient needs. Moreover, it is important to keep in mind

that many female athletes underestimate caloric intakes (56). Athletes also tend to either under-report drug and supplement use in order to conceal behaviors or over-report substances that may impress others (57). Supplement use may be beneficial to those athletes who do not consume adequate energy or nutrient intakes.

Supplements

Athletes take supplements in order to enhance performance or avoid sickness. Weight loss, weight gain, increased focus, quicker recovery, and other physiological benefits of various supplements are sought for improved performance. In a study of 162 collegiate female varsity athletes, 60.1% cited the reason for using supplements was for “good health” (58). This same study reported that 35.8% used a multivitamin/mineral supplement with iron, 31% used a vitamin C supplement, 12% used an amino acid/protein supplement, 58% consumed coffee, and 50% consumed sports bars (58). The most common supplements used by athletes and the general college population are multi-vitamin/mineral supplements, vitamin C, and iron (58, 59).

Many (non-athlete) American adults take vitamins, minerals, botanicals and other dietary supplements, though the prevalence of use varies by age, gender, race and ethnicity (60). While little is known about supplement use in the college-age and young adult population, survey results show a strong association between high levels of physical activity and use of supplements (60). Studies of college and university students report prevalence of herb and dietary supplement use to be 26-79% (61-63). Women are more likely than men to use supplements and 31% of females 18 to 24 years of age, use vitamin or mineral supplements (64). Sixty-two percent of female college students and 57-65% of female athletes report taking vitamin/mineral supplements (59, 65).

Various sources and types of protein supplementation are commonly used by athletes in attempts to increase lean muscle mass and strength (66). Protein supplementation by those athletes not consuming enough dietary protein (1.2 – 1.4 g protein/kg body weight/day for endurance athletes is recommended) may be beneficial to prevent muscle and/or bone loss due to inadequate energy intake (66). Protein intakes of 69 ± 19 g/day were found to be significantly correlated to aBMD of the total hip, femoral neck, and trochanter in a cross-sectional study of 107 Caucasian females aged 18 years (67).

Athletes and non-athletes consume one of the most commonly used ergogenic aids, caffeine, in similar amounts. Caffeine, at levels of 250-700 mg, has been shown to improve cycling and running performance and decrease recovery times but may not have a significant effect on short-burst activity (67). Lower doses of caffeine may improve hand steadiness, reduce fatigue, and increase feeling of alertness (67). Though caffeine consumption is proposed to affect aBMD by increasing calcium excretion, a cross-sectional study of 177 Caucasian college-aged females found no relationship between caffeine intake and femoral neck or lumbar spine aBMD even after controlling for covariates (68).

Though use of ergogenic aids is widespread among NCAA division I athletes, the most commonly used supplements that may have the largest impact on bone health are multi-vitamin/mineral supplements because of the additional amount of calcium and vitamin D that are ingested per the supplement. Though many dietary factors influence bone strength, two important nutrients for bone development that will be discussed below are calcium and vitamin D. Calcium and vitamin D help maintain blood calcium homeostasis and regulate hormonal activity and therefore have the potential to influence aBMD (69).

Calcium

Bone tissue is the calcium reservoir where 99% of body calcium is stored (69). If dietary calcium is inadequate for an extended period of time, serum calcium levels will decrease and hormones will stimulate increased resorption of calcium from the bones to release calcium into the blood to maintain circulating plasma levels. To prevent calcium resorption and possible bone loss, the adequate intake (AI) of 1000 mg/day should be consumed in this young adult age group (70). Calcium intakes of 1300 mg/day can be beneficial in young adult females, but amounts in excess may be excreted (71). In 1997, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes reported that the median dietary calcium intake for 19-30 year old females was 647.2 ± 46.3 mg/day (72). Studies of young adult females report typical dietary calcium intakes to be approximately 600 mg/day (55) and 753 ± 63 mg/day (53). Mean calcium intakes for non-athlete college-aged females suggest that, on average, this population group consistently fails to meet the AI for calcium (72).

As for collegiate athletes, according to an investigation of 59 female Euro- and African-American NCAA division I athletes, dietary calcium intakes averaged 898 mg/day (73). In various reports of mean dietary calcium intakes of female runners, NCAA division I cross-country runners reported 605 mg/day (73); 91 young adult competitive runners consumed 1428 ± 115 mg/day (54); and 26 young adult distance runners consumed 1150 ± 859 mg/day (55). Collegiate gymnasts were estimated to consume 683 ± 58 mg/day (53). According to the previously mentioned reported dietary intakes of calcium, it seems that, on average, gymnasts, runners, and non-athletes consistently consume below the current recommended adequate intake (AI) of 1000 mg/day of calcium for adult women over 19 years of age (70).

Although young adult females consume less than optimal dietary calcium, the association between calcium intake and bone mass among premenopausal females is relatively weak (correlation coefficient $R = 0.12$) according to a recent meta-analysis (74). Most studies that find any correlation between calcium intakes and bone mass include subjects who are growing rapidly in childhood or adolescence (75-77), provide calcium supplements containing more than the AI of calcium (55), or include subjects consuming extremely low amounts of calcium (78).

The results of cross-sectional studies on the effect of dietary calcium on bone mass have been mixed (75, 79-81). Many cross-sectional studies of college-aged females that assessed the effects of dietary calcium intakes on aBMD show no significant correlation between dietary calcium intakes and aBMD. In a cross-sectional study of 115 females aged 18 years, calcium intakes of 730 ± 324 mg/day had weak correlations with aBMD at all bone sites measured except at the trochanter site ($r=0.19$) (67). In a study of 180 Japanese college females (20-23 yrs), very low self-reported calcium intakes (411 ± 196 mg/day) had no significant correlation to lumbar spine aBMD nor irregular menstrual cycles (82). Even in a study with higher self-reported dietary calcium intakes (1074 ± 463 mg/day) which averaged around the AI, no effect was found between calcium intake and lumbar spine or hip aBMD of 107 healthy college-aged Caucasian females (83).

Studies of collegiate athletes and very physically active college-aged females show similar correlations between dietary calcium intakes and aBMD. A cross-sectional study of 18-24-year-old female college athletes (runners, swimmers, and controls) found no significant correlation between calcium intake and aBMD (84). One prospective study of collegiate gymnasts found a significant negative correlation between calcium intake (within the year) and aBMD for gymnasts but no significant relationship between calcium intake and BMD in controls

(53). In a 3.6 year study of physically active college-aged female military cadets, milk consumption and total calcium intake had no effect on aBMD at the lumbar spine or hip (83). Taken together, these cross-sectional and prospective studies suggest that usual dietary calcium intakes in college-aged females (especially athletes), even if slightly lower than the recommendations, may not affect bone mass negatively.

Various longitudinal studies of non-athletic college-aged females did not find any significant associations between dietary calcium and aBMD. In a 10-year longitudinal study of 80 community dwelling 17-22 year old females, daily calcium intakes (ranging from 500-1900 mg/day) were not significantly associated with aBMD changes (85). However, in a 5-year prospective study of college-aged women, after adjusting for protein intake, self-reported dietary calcium intakes had a strong influence on aBMD (35). Discrepancies in the above studies may be due to site specificity. For example, a 1996 review summarized that high calcium intakes (>1000 mg/day) significantly benefited lumbar spine aBMD but not radial aBMD (86).

Aside from dietary calcium intake, various calcium supplementation trials show that calcium supplementation has a modest benefit on young adult bone. In a double-blind, placebo controlled, randomized trial, one year of supplementing 19- to 27-year-old female distance runners with 1000 mg/day calcium or placebo tablets showed that calcium supplementation prevented aBMD loss at the femoral mid-shaft (0.1% vs. -1.8% change in aBMD, for calcium vs. placebo, respectively), but found no significant differences at the hip or spine (55). A similar study of young female distance runners found supplementing 1500 mg calcium/day significantly maintained tibial mid-shaft aBMD but found no significant difference at the hip or spine (87). Other calcium supplementation studies report similar effects on aBMD in young adults, which suggests that calcium intakes above the recommended adequate intake (1000 mg/day) may not

increase bone mass or aBMD (88). Therefore, calcium supplementation may help prevent bone loss but may not contribute to additional bone accrual in college-aged females.

Adequate calcium intakes are beneficial for aBMD in every age group; however, calcium intake seems to have a more significant effect on aBMD gains during the teen years than the adult years (53). Adequate calcium intake may be more important for females not engaging in weight-bearing physical activities—as evidenced by the negative correlation between calcium intake and aBMD in college gymnasts but no significant correlation in controls (53). Perhaps mechanical loading and weight-bearing exercise have a greater influence on bone growth than optimal calcium intakes in this age group (53). The section, “Physical Activity” later in the chapter discusses this possibility in further detail.

Vitamin D

The estimated dietary intake of vitamin D in 19-50 year old females is 3.8 micrograms/day (89), which is well below the recommended adequate intake (AI) of 5 micrograms vitamin D/day for 19-30 year old women (70). Using food consumption surveys, the second National Health and Nutrition Examination Survey (NHANES II) found that the median dietary vitamin D intakes of young women were approximately 2.9 micrograms (114 IU)/day, with a range of 0 to 49 micrograms (0 to 1,960 IU)/day (90). Female adolescents and young adults have the lowest intakes of vitamin D within the US population, which puts these populations at risk for suboptimal levels of circulating vitamin D, which could lead to negative health consequences (89).

Vitamin D is a fat-soluble vitamin essential for intestinal calcium absorption and for maintaining adequate concentrations of serum calcium and phosphorus. Derived from sunlight exposure (D3), the diet (D2 or D3), or through supplementation (D2 or D3), vitamin D is crucial

for bone metabolism (89, 91). Season, latitude, race, and age also affect one's ability to synthesize vitamin D. UVB exposure is necessary for vitamin D synthesis in the skin. Five to thirty minutes of mid-day sun exposure twice/week, without sunscreen, are necessary for sufficient vitamin D synthesis. Especially in higher latitudes and in the winter months (October-March) when sun exposure is less frequent, vitamin D deficiency may result in lower aBMD among adolescents and young adults (92).

In adults, poor vitamin D status results in decreased calcium absorption, elevated levels of parathyroid (PTH) hormone and is related to low bone mass and increased risk of fracture (93, 94). Though the relationships between serum 25(OH)D and bone are more clearly documented in adults, the effects of vitamin D on attainment of PBM in growing children is still unclear (91). While there are reports of vitamin D supplementation improving aBMD in adults (93, 95-97), studies of adolescent and post-pubertal females report either no correlation between vitamin D status and bone mass (91, 98), a significant negative relationship between 25(OH)D on BMC gains (99), or modest improvements in bone mass with supplementation, but usually in those who are vitamin D deficient (100).

It has been proposed that physical activity has a greater effect on bone growth than diet—even when calcium and vitamin D intakes are sub-optimal (101). Due to the many lifestyle factors influencing bone it is important to remember that “nutrition alone does not influence muscle or bone in a dose-dependent manner” (26, 69). Dietary calcium and vitamin D are not the only nutritional factors affecting bone growth in late puberty and early adolescence. Other factors, such as hormonal status and oral contraceptive use are important factors as well.

Hormones

Parathyroid hormone, vitamin D, and calcitonin regulate serum calcium levels, but bone growth is mainly regulated by growth hormone (GH), insulin-like growth factor-1 (IGF-1), and sex hormones. Production of GH is increased during stress, sleep, exercise, and an anabolic metabolic environment—with highest circulating levels immediately following meals or exercise and during slow-wave sleep (6). GH is the primary regulator of linear bone growth in childhood and adolescence (102) and is mediated through the actions of IGF (103). GH secretion, which declines with age (at a rate of 14% per decade) is one reason for low serum IGF levels often observed in elderly populations (104). Serum IGF-I concentrations have been shown, in some studies, to be directly related to aBMD (105). Circulating IGF affects the action of GH and bone remodeling by various mechanisms not yet fully understood. It has been observed, however, that IGFs are essential to regulating the bone remodeling process in response to increased bone resorption (19)

In the post-pubertal years, sex steroids and GH are key regulators of bone growth. The predominant sex hormone in men is testosterone and in women is estrogen. Androgens and estrogens are present in both males and females, but because estrogen stops being produced post-menopause it is a very influential hormone for women's bone health (6). Estrogen inhibits bone resorption and indirectly retains calcium resulting in decreased bone turnover (6). In a recent cross-sectional study of 242 healthy women aged 30-40 years, neither circulating concentrations of estradiol, testosterone, nor progesterone were independent predictors of lumbar spine aBMD or total hip aBMD (106). Studies of hormones and bone focus on the effects of using oral contraceptives (OC), which are hormonal preparations that may contain combinations of estrogen and progestin or progestin alone.

Oral Contraceptives

Oral contraceptive (OC) use is common among college-aged females—athletes and non-athletes alike—for the management of menstrual irregularity, menstrual cramps, heavy menstrual bleeding, or birth control (66). A large study of 938 elite athletes and 900 age-matched non-athlete controls reported a higher percentage of athletes (40.2%) taking OCs than controls (27.5%) (107). In a particular sport, artistic gymnastics, 33% of gymnasts reported OC use where as 63% of controls reported using OCs (108). Many female athletes use OCs in order to regulate the timing of their menstrual cycles and avoid interface with important competitions. Bloating, fluid retention, breast tenderness, and menstrual cramps associated with the menstrual cycle can affect athlete performance (109), and in one study, these menstrual cycle effects negatively affected the performance of 50% of the athletes (110). In another study, six post-menarchal swimmers swam the fastest 100-yard freestyle times during menses and the slowest times during the premenstrual phase (111), which further supports the idea that menstrual status can affect performance. Water retention during the premenstrual phase and water reduction during menses was the probable cause for the difference in performance (111). While a significant number of athletes and non-athletes use OCs for various reasons, the effects of OCs on bone mass are unclear.

Oral contraceptive use has been associated with reduction of endogenous sex steroids, which is significant because both estrogen and testosterone can affect bone metabolism and remodeling. Estrogen suppresses bone remodeling as evidenced by the increased rate of bone loss in postmenopausal women that accompanies the decreased production of estrogen (112, 113). Thus, estrogen-containing OCs (such as ethinyl estradiol) may be protective against bone loss, whereas progesterone-containing oral contraceptives may work oppositely.

Since the estrogen and progestin content has been reduced in many OCs in recent years, many studies have focused on the effects of OCs on bone density (114). In a partially randomized cohort study of 71 women aged 22-34 years, after one year of taking OCs, no significant difference in aBMD was found between those taking OCs and controls (115). In a cohort study of 18-39 year old women taking estradiol, the OC users did not have significantly different aBMD change or in aBMD over three years (114). Many other studies of young adult women found no significant differences between OC users and controls (116-119). A systematic review of the literature by Martins, et al (120), from 1966-2005 on OC use in adolescents and young adult women, concluded that the research is unclear as to whether OC use prevents young women (<23 years of age) from achieving their peak bone mass, and that adult women who use OCs have similar aBMD to non-users (120).

In athlete populations, OC use may have a protective effect on amenorrheic athletes. In one cross-sectional study of collegiate long distance runners and fracture rate, runners who had never used OCs were more than twice as likely to experience a stress fracture than those who had used OCs for over a year (121), suggesting that OCs have a beneficial effect on bone. The effect of OC use on bone in gymnasts is still unclear and controversial; however, the consequences of delayed menarche and primary and secondary amenorrhea on bone are well documented (108, 122, 123).

Menarchal Status

Research strongly supports the notion that, on average, amenorrheic/oligomenorrheic athletes have lower aBMD than eumenorrheic controls and that the relationship between menstrual irregularity and low aBMD exists within the female athlete triad (11, 124-134). Amenorrhea is more prevalent in athletes due to the increased energy expenditure of exercise

and/or the pressure to obtain an optimal body weight which may lead to low energy availability, menstrual irregularities, and subsequent loss of aBMD—the component of the female athlete triad (135). One study looking at those female athlete triad components (disordered eating, menstrual irregularity and BMD) in young adult female runners found differences in aBMD between eumenorrheic and abnormally menstruating runners (54). Of the 91 young adult competitive runners, abnormally menstruating runners (n=36) had lower aBMD than eumenorrheic runners (n=58) at the lumbar spine (-5%), hip (-6%) and total body (-3%) after accounting for weight, % body fat, eating disorder inventory score, and age at menarche (54). The average number of cycles per year was 8.25 ± 0.3 for all 91 runners (54). Other studies of young adults also report the prevalence of abnormal menstrual cycles in competitive runners to be 50% eumenorrheic, 39.3% oligomenorrheic, and 10.7% amenorrheic runners compared to 100% eumenorrheic controls in one study (136); 64% eumenorrheic, 26% oligomenorrheic and 10% amenorrheic runners in another study (54).

Female gymnast populations also exhibit a higher prevalence of menstrual dysfunction than non-athletes (137). Primary amenorrhea has been found in 15-20% of elite female gymnasts (131, 138, 139) and secondary amenorrhea in 40-60% of elite female gymnasts (53, 131). Interestingly, despite menstrual irregularities, late adolescent and collegiate gymnasts have greater aBMD than normally active females, runners and volleyball players (137). This suggests that high-impact exercise producing ground reaction forces of up to 11 times body weight, like gymnastics, could counterbalance the detrimental effects of hypoestrogen seen with menstrual irregularities (137). The percent change in aBMD, even when stratified by menstrual status or oral contraceptive use, did not differ at any clinically relevant bone site ($p>0.05$) in an 8-month longitudinal study of 18- to 23-year-old gymnasts, runners and controls (140).

Though many studies exclude subjects who are amenorrheic for more than 3 or 6 months, some studies of athletes have used subjects regardless of menstrual status to increase sample size (increase number relative to normal population). Despite the prevalence of irregular menstruation in athletes, studies have shown no significant differences between aBMD of amenorrheic, oligomenorrheic, and eumenorrheic athletes.

Age at Onset of Menarche

Delayed menarche may precede later menstrual dysfunction and/or affect the full potential of bone mass gains. Generally, athletes achieve menarche later than controls. One study showed over half of the athlete group started menarche after age 14 years whereas only 14.9% of the non-athlete group started menarche after age 14 years (141). Non-athletic females have been found to start menarche around age 12.7-13.2 years (83, 136, 142). In a study of 180 college females aged 20-23 years, later onset of menarche increased the likelihood of irregular menstrual cycles and is inversely related to lumbar spine aBMD (82). Conversely, age at menarche in 107 Caucasian healthy college-aged females was not correlated with hip or lumbar spine aBMD (83).

In a study of 91 competitive young adult runners, eumenorrheic runners (n=58) started menarche at 12.6 ± 0.2 years, whereas the runners with menstrual irregularities (n=33) started significantly ($p < 0.0001$) later, at 13.8 ± 0.2 years of age (54). Collegiate runners reported a mean age at menarche of 13.5 years, approximately one year later than controls (84). Gymnasts are also found to start menarche later than controls. On average, gymnasts start their menstrual cycles at 15.6 years of age, which is significantly later than the typical reference group, 13.2 years of age (142, 143). Gymnasts also start menarche at a significantly ($p < 0.05$) older age than other athletes (swimmers and tennis players) (143, 144).

Despite consistent differences in onset of menarche among athletes versus non-athletes, the majority of studies have shown age at menarche in athletes and years since menarche to be insignificant and/or not correlated to aBMD (145). This suggests that, similar to the effect of inadequate diet on achievement of PBM, perhaps mechanical loading and weight-bearing exercise has a greater influence on bone growth than age of menarche. Like inadequate calcium intakes, perhaps the negative effects of hormonal imbalances on bone may also be alleviated or supplemented by physical activity (53). The next section, “Physical Activity,” discusses this possibility in further detail.

Physical Activity and Bone

Physical activity is one of the most influential contributors to bone strength (26). Bone gains via physical activity are maintained into young adulthood (23-30 years of age) (146), but not all exercise is effective for optimizing peak bone mass (147). The progression of knowledge about the benefits of various types of physical activity on bone have been demonstrated in many cross-sectional, prospective, observational, and intervention studies. Early studies of athletes gave us one of the first indications that weight-bearing loading improves bone mass (148, 149). Studies of astronauts who lost significant bone mass during zero-gravity space flight added to the growing evidence that bone requires weight-bearing loading forces in order for bone remodeling to occur (46). After discovering the importance of weight-bearing exercise on bone, studies comparing dominant and non-dominant limbs in tennis players and golfers discovered that impact forces, not just weight-bearing forces, influence bone. In the late 1990’s limb-specific studies on tennis players showed 20% increases in muscle and bone mass only in the dominant playing arm—suggesting that increased muscle mass has a positive effect on bone mass but not necessarily bone density (150). A study of professional male golf players showed a 6% higher

BMC in the dominant arm compared to controls despite an insignificant difference in the amount of muscle mass between arms (151). These findings helped demonstrate that it is not purely muscle mass or joint-reaction forces that increase aBMD but the actual impact of the forces that stimulate bone modeling and remodeling.

In the 1990's, cross-sectional studies began to show that weight-bearing impact-loading activities, such as gymnastics and running, may increase aBMD more than active loading sports, such as swimming or cycling (140, 141, 152). More specifically, longitudinal studies showed that it is not the frequency but the magnitude of the loads imparted on the limb being used that increase aBMD the most (140, 141, 153). Studies of gymnasts showed that the impact forces generated by gymnasts are between 5-10 times (154-156) or up to 12 times their body weight (157). Compared to running, which imparts forces 3-5 times body weight, gymnasts have hip and lumbar spine aBMD values up to 30-40% higher than those of long-distance runners (131).

Different activities and sports place varying forces on bone causing surface- and site-specific responses. Activities with high magnitude ground reaction forces at varying angles are most effective for increasing aBMD. In contrast, duration and frequency of exercise may be less effective than the magnitude of the forces applied to bone (158, 159). Gymnasts, for example, endure many axial forces whereas tennis players experience a greater proportion of torsional forces (47). Running elicits less ground reaction forces per step but at a higher frequency. Gymnastics yields fewer impacts than running but the magnitude of the impacts are higher and the variation of strain angles is greater than running. Thus, gymnasts tend to have higher aBMD than runners (160).

Various jumping intervention trials provide further evidence that high impact load activities are optimal for bone mineral accrual during growth (161). In one jumping intervention

study, participants jumped for 10 minutes, 3 days/week (maximum of 100 jumps on a 24 inch box) for 7 months and aBMD was measured for 8 years. Jumpers had 3.6% greater bone mass immediately after the intervention (7 months after baseline) compared to controls. After 8 years, jumpers had 1.4% greater total hip aBMD, showing that although benefits of exercise are not fully maintained when activity stops, some benefits of jumping are maintained (162). After a six month jumping intervention of 36 college-age (aged 19-21 years) female students, jumpers had significant increases in aBMD at the femoral neck and lumbar spine after 6 months compared to no change in aBMD in the control group (163). By 2004 there was enough evidence for the American College of Sports Medicine to publish a position stand on specific jumping exercises for optimizing bone growth and maintenance (164).

Recent cross-sectional studies of young adult females show that runners have higher aBMD than non-runners. Duncan et al (145) performed a cross-sectional study of cyclists, runners, swimmers, triathletes, and controls aged 15-18 years ($n=15$ per group) and found the following significant ($p<0.05$): runners had greater total body aBMD and femoral neck aBMD than both swimmers and controls; runners had greater leg aBMD than swimmers, cyclists and controls; and runners had greater lumbar spine aBMD than controls (145). In a cross-sectional study of 65 runners 19-50 years of age, runners who also did resistance training more than twice/week had greater lumbar spine aBMD than those who performed resistance training less than twice/week ($p<0.01$) (165). A cross-sectional study of collegiate runners ($n=21$), swimmers ($n=22$) and age-matched non-athletic female subjects showed no significant differences in aBMD among the three groups. However, total body aBMD and femoral neck aBMD were positively correlated with weight-bearing activity but not with non-weight-bearing activity. Also, lumbar spine aBMD was higher in subjects who had previously used OCs (84).

A recent prospective study over 15 years of 82 females split into 3 groups according to activity level found that aBMD benefits of physical activity are maintained into adulthood (146). A 12 month longitudinal assessment of power athletes, endurance athletes and age-matched non-athlete controls, aged 17-26 years, found that bone mineral accrual continues into the young adult years (141). In this particular study, both athlete groups had significantly greater aBMD gains at the lumbar spine than controls (141). In a shorter longitudinal study over 6 months, gymnasts had a 1.3% increase in lumbar spine aBMD, whereas the control group saw no change (166). Over 24 months, a

small study of 8 collegiate gymnasts (from age 19 to 21 years) observed a 4.3% increase in aBMD at the lumbar spine but no overall change at the hip (167); however, there was no control group. In a 3.6 year longitudinal study of 164 college-age women active in the Navy, the percent

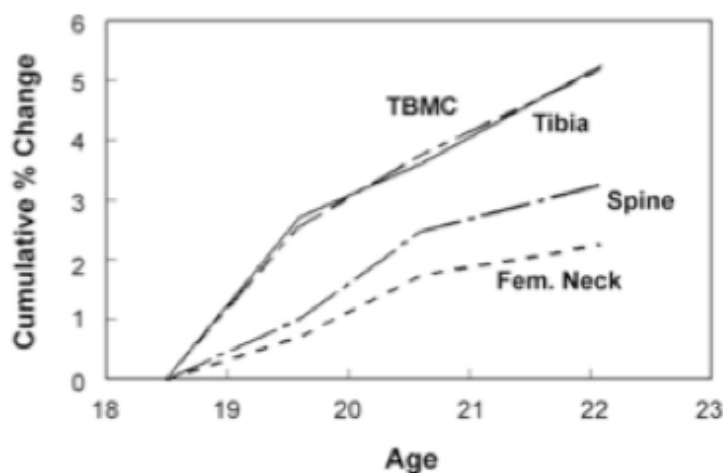


Figure 2.3. Cumulative percent (%) change in total body BMC, lumbar spine, femoral neck, and distal tibia aBMD over 3.6 years. Adapted from Drake et al (168).

change in lumbar spine aBMD and femoral neck aBMD was significant ($p < 0.001$) over the college years ($3.27 \pm 0.35\%$ and $2.26 \pm 0.39\%$, respectively); however, there was no control group (figure 2.3) (168).

Most studies focus on the effects of physical activity on bone in childhood, adolescence, and menopause while little research exists on the young adult years. The early twenties are presumably a time when the final additions to aBMD can be made, especially at the lumbar spine, before bone mineralization peaks. This could also possibly be a “catch up” period after adolescence—when diet and physical activity may not have been adequate for optimal bone accrual (169). It may be very reassuring to discover that the college years and early 20s present an extended window of opportunity to maximize bone consolidation. While the importance of this time frame of life is promising to aBMD accrual, the literature in this area is lacking (169). The effects of rigorous physical activity during this stage of life may positively affect how much aBMD is gained before PBM is reached, and thus decrease the risk of osteoporotic fractures later in life.

A systematic review of the literature on exercise and bone mass of the femoral neck and lumbar spine from 1966-1997 concluded that exercise slows bone loss at the lumbar spine and “probably the femoral neck” in postmenopausal women, but more studies are needed to reach a firm conclusion in the pre-menopausal population (170).

Summary

In summary, during growth, bone is very responsive to factors influencing bone mineral accrual including diet (supplement use, calcium, and vitamin D), hormones, menarchal status, and physical activity. The effects of physical activity on aBMD in childhood and adolescence show that high-impact-loading activities are beneficial and that bone gains during these formative years can be maintained into young adulthood (146, 171). Cross-sectional assessments of the effect of sports participation on bone during the young adult years (131, 141, 172-174) are consistent with research of younger populations—that females participating in high-impact

exercises, like artistic gymnastics, tend to have greater bone mass than those participating in non-weight-bearing activities (175-179). Bone is still mineralizing during the young adult years, as evidenced by the gains seen in the few studies following females throughout all 4 college years (35, 146, 168); however, to our knowledge, there is not one study assessing the effects of sports participation (artistic gymnastics and running) on aBMD throughout the college years.

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CHAPTER 3

BONE MINERAL DENSITY IN COLLEGE FEMALE ATHLETES AND NON-ATHLETES:

A THREE YEAR STUDY

Maust, C.V., Laing, E.M., Hall, D.B., Pollock, N.K., Taylor, R. G., Baile, C.A., Lewis, R.D.

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Maust, C.V.¹, Laing, E.M.¹, Hall, D.B.², Pollock, N.K.³, Taylor, R. G.¹, Baile C.A., Lewis R.D.¹

¹Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA

²Department of Statistics, The University of Georgia, Athens, GA, USA

³Department of Pediatrics, Prevention Institute, Medical College of Georgia, Augusta, GA, USA

Running Title: GYMNASTS, RUNNERS, YOUNG ADULTS

Emails:

cmaust@uga.edu

emonk@uga.edu

danhall@uga.edu

npollock@mail.mcg.edu

rgildea@uga.edu

cbaile@uga.edu

rlewis@fcs.uga.edu

Correspondence and reprint requests should be addressed to: Richard D. Lewis, PhD., Room 279 Dawson Hall, Department of Foods and Nutrition, The University of Georgia, Athens, GA 30602. Phone: 706-542-4901. Fax: 706-542-5059. Email: rlewis@fcs.uga.edu

ABSTRACT

The degree to which different sports can affect bone mineral changes throughout the college years is unknown. The purpose of this study was to determine the effects of sports participation during the college years (36 months; freshman through senior year) on changes in areal bone mineral density (aBMD) in female artistic gymnasts (GYM; N=37), cross-country runners (RUN; N=28), and non-athlete controls (CON; N=69). Body composition and total hip, femoral neck, trochanter, and lumbar spine bone measurements of GYM, RUN, and CON were determined using dual X-ray absorptiometry (Delphi A; S/N 70467; Hologic Inc., Bedford, MA). Data were analyzed using linear models with PROC GLM on Statistical Analysis Software version 9.1 (SAS, Cary, NC), and fixed effects statistical methods were performed using ordinary least squares linear regression. Mean percent changes in body composition over 36 months were not significant, however at baseline, GYM and RUN had significantly lower body fat % than CON. A significant decline in total hip and femoral neck aBMD was observed in CON, but not in GYM or RUN ($p<0.0001$). All groups had a significant decline in trochanter aBMD ($p<0.0001$). Lumbar spine aBMD increased significantly in GYM ($p<0.05$) over 36 months, but not in RUN or CON. These results suggest that participation in weight-bearing sports during the collegiate years, a time associated with peak bone mass, has a beneficial and/or protective effect on total hip, femoral neck and lumbar spine aBMD, but not necessarily the trochanter.

Introduction

The beneficial role of physical activity and weight-bearing exercise on bone gains has been clearly documented in cross-sectional (1) and longitudinal (2, 3) studies of growing children. Furthermore, studies suggest that the benefits of physical activity on bone gained during childhood and adolescence are greater with high vs. low impact activities and are maintained into young adulthood (4, 5). Since bone changes are less dramatic in the young adult years than during puberty, studies on physical activity and bone have been focused on the rapid growth phase and less on young college-aged adults.

Cross-sectional studies of young adult athletes participating in various sports show that athletes participating in sports with more high impact and weight-bearing loads have increased bone mass and strength (6-10). For example, weight-bearing activities such as volleyball, tennis, running, gymnastics, and power lifting benefit bone mass development (6, 11) whereas non-weight-bearing activities (e.g., swimming and cycling) consistently show no significant difference in areal bone mineral density (aBMD) vs. controls (12-14). Collegiate artistic gymnasts are known to have a significantly higher aBMD at the total body, total hip, femoral neck, and lumbar spine than non-athlete controls (15). A small study of 8 collegiate gymnasts observed a 4.3% increase in aBMD at the lumbar spine but no overall change at the hip over 24 months, from age 19-21 years (16); however, there was no control group. Over an 8-month prospective study of collegiate gymnasts, runners and controls, lumbar spine aBMD gains of the gymnasts were significantly greater than both runners and controls ($2.8 \pm 2.4\%$ vs. $-0.2 \pm 2.0\%$ vs. $0.7 \pm 1.3\%$), respectively (14). At the femoral neck, runners experienced a significant loss of aBMD while gymnasts experienced a significant ($p < 0.05$) gain over 8 months (14). Significant changes in aBMD over 8 months during the college years suggest that the potential for additional

bone mineralization still exists. A longer-term prospective study assessing the change in bone throughout the college years found significant increases in all bone sites except the trochanter (17). In this study, 164 highly physically active Caucasian females had significant ($p<0.0001$) increases in aBMD at the hip (2.26%), lumbar spine (3.27%), and total body BMC (5.25%), and a non-significant ($p>0.05$) decline in trochanter aBMD (-0.6%) over 3.6 years (17). However, it is not known in this study if high levels of physical activity led to the changes because there was no control group (17). To our knowledge, the two aforementioned prospective studies are among the few studies focusing on the changes in aBMD in physically active females during the college years.

In order to determine the potential for sports participation to modify bone in young adulthood specifically, the current study was performed to assess changes in aBMD at the total hip, femoral neck, trochanter, and lumbar spine over 36 months in competitive artistic gymnasts and cross-country runners, as well as healthy, non-athlete females. We hypothesized that aBMD of the lumbar spine, but not the hip, would increase in the gymnasts, cross-country runners, and non-athlete controls over 36 months with gymnasts experiencing the greatest increases.

Materials and Methods

Study Design

Female NCAA Division I collegiate artistic gymnasts (GYM; $n=37$), cross-country runners (RUN; $n=28$), and non-athlete controls (CON; $n=69$) participated in this 36 month prospective observational study. Data were obtained annually for the athlete groups and at baseline and 36 month follow-up for the control group. Data for all groups were collected in the fall season. The same lab technician conducted all scans and analyses using the same bone densitometer. The Institutional Review Board for Human Subjects at UGA approved all

procedures for this study, and written informed consent was obtained from each subject. All testing took place at The University of Georgia Bone and Body Composition Laboratory in Athens, GA, USA.

Participants

All subjects were Caucasian female college students and at baseline, were aged 18.1 - 19.4 years. Because the CON group participants had participated in another research project, exclusion criteria from that project were applied to the current project. Women in the CON group who had experienced significant weight gain or loss in the past 6 months ($\pm 10\%$ initial BW), consumed vegetarian diets, participated in NCAA Division I athletics, had diagnosed eating disorders, irregular menstruation (less than 4/6 periods in the last six months), and those taking medications or herbal supplements known to affect body weight were excluded. Other than race, there were no exclusion criteria for either athlete group.

The practice routines as described by the coaches for GYM included, on average, 3 hours of gymnastics 4 days/week plus 3 days/week of weight training and 3 days/week of cardiovascular conditioning in the fall. During the competition season (spring), GYM practiced 3 hours/day for 3 days/week plus 2 days/week of weight training, and 3-5 cardiovascular workouts/week. Cardiovascular workouts consisted of riding a stationary bike or using the elliptical machine (no running was allowed). A 5 hour competition each week added to the total gymnastic training time in the spring.

Cross-country runners, on average, ran approximately 8 miles/day for 6 days/week for a total range of 45-80 miles/week. This group also had two 1-hour weight lifting sessions per week focusing on light weight and high repetitions for upper and lower body and core strengthening exercises. In the event of injury or other need for cross training, RUN would

swim, ride a stationary bike, use an elliptical machine or underwater treadmill, instead of running.

Based on the 7-day physical activity recall questionnaires, the CON group, on average, spent 16.1 hours/day doing light physical activity, 0.21 hours/day participating in moderate physical activity, 0.02 hours/day in hard physical activity, and 0.16 hours/day doing very hard physical activity at baseline. The CON group, therefore, spent an average of 0.39 hours/day doing moderate to very hard physical activities. Compared to GYM, who spent an average of 3 hours/day doing hard to very hard physical activity, and RUN who spent an average of 1.2 hours/day doing hard to hard to very hard physical activity, CON were significantly less physically active than both GYM and RUN.

GYM data were collected from August 2002-August 2009, RUN data were collected from August 2004 - August 2009, and CON data were collected August 2005-August 2008. In order to capture as much data as possible from our participants, data from athletes who were still currently competing were used as well as data from currently retired athletes who had already finished their four years of eligibility. Due to the intense nature of competing in collegiate gymnastics and cross-country running, some athletes inevitably quit the sport during their collegiate career. Due to these two factors (i.e., current athletes without four years of data and athletes who quit mid-career), the number of GYM and RUN subjects used varied over time. In this population, 11 GYM (29.7%) and 5 RUN (17.9%) quit. Of these 11 GYM who quit, 4 GYM quit after only 1 year of competing, 5 GYM quit after 2 years of competing, and 2 GYM quit after 3 years of competing. Of the 5 RUN who quit, 4 RUN quit after 2 year of competing and only 1 quit after 3 years of competing.

The numbers of current athletes who had not yet finished their 4 years of eligibility are 10 for GYM and 12 for RUN. Of the 10 GYM, 4 are currently in their 1st year, 4 in their 2nd year, and 2 in their 3rd year of eligibility. Of the 12 RUN, 5 are in their 2nd year and 7 in their 3rd year. Table 1 describes the number of subjects in each group from baseline to 36-month follow-up. For further explanation and an example, current 3rd year junior athletes only have 3 measurement points, current 2nd year sophomore athletes only have 2 measurement points, and so forth. In the GYM dataset, due to current year of eligibility and the number of those who quit, 8 GYM have 1 data point, 9 GYM have 2 data points, and 4 GYM have 3 data points. Likewise, 9 RUN have 2 data points and 9 RUN have 3 data points.

Anthropometry

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Novel Products Inc, Rockton, IL). Weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Height and weight measurements were made twice at each visit and average measurements were used in the analysis. If two measurements differed by greater than 1.0 cm or 0.1 kg, three measurements were taken and the two closest values were averaged.

Body Composition/Bone Density

Body composition variables [fat mass (kg), fat-free soft tissue mass (kg) and percentage body fat] and bone outcomes of the total body, non-dominant hip (total hip, femoral neck, trochanter), and lumbar spine [aBMD (g/cm²), bone mineral content (g) and bone area (cm²)] were measured using dual energy X-ray absorptiometry (DXA; Delphi A; S/N 70467; Hologic Inc., Bedford, MA). The same technician analyzed all scans using Hologic software, version 11.2. Quality assurance for total body DXA measurements was performed via calibration against the manufacturer's 3-step soft-tissue wedge (model TBAR; SN 2275) and anthropomorphic spine

phantom (model DPA/QDR-1; SN 9374) composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). Calibration of the DXA against a spine phantom and radiographic uniformity tests were conducted weekly. A coefficient of variation of 0.36% was observed from 648 scans of the spine phantom over a 3-year period. For determination of measurement reproducibility, one-way random effects model, single measure intraclass correlation coefficients (ICCs) were calculated in 10 females aged 18-30 years, scanned twice using the Delphi-A instrument over a 7-day period for fat mass, fat-free soft tissue mass, and % body fat (all $R \geq 0.87$) and for BMC and aBMD of the whole body, LS, and hip (all $R \geq 0.96$).

Statistical Analyses

Trend with age between groups were analyzed using the SAS statistical package [SAS System for Windows, v 9.1 (SAS Institute, Cary, NC)]. Fixed effects methods using linear models with PROC GLM and ordinary least squares linear regression were performed, thus accounting for between subject differences. One-way ANOVA was used to calculate baseline differences (means and standard deviations) and mean percent change for each group. An alpha level of 0.05 identified statistically significant differences within and between groups.

Results

Participant Characteristics

Participant characteristics at baseline (freshman year) and 36-month follow-up are presented in Table 3.2. The age of subjects ranged from 18.1 – 19.4 years at baseline. Baseline height was significantly different between groups, with GYM shorter than RUN and CON. Both GYM and RUN weighed significantly less and had significantly lower body fat % than CON (123.2 ± 14.2 and 123.0 ± 12.1 vs. 132.5 ± 17.6 lbs) and (19.7 ± 3.1 and 20.8 ± 3.1 vs. 28.3 ± 4.2

%), respectively. Areal BMD at all sites (total body, femoral neck, total hip, and lumbar spine) except the trochanter was significantly higher in the GYM than both RUN and CON.

In controls, at the 36-month follow-up, only light physical activity decreased (by 1.9 hours/day), whereas each of the other three more intense levels of physical activity increased significantly. Estimated energy expenditure for this group also increased significantly over 36 months (2089 ± 329 kcal/day at baseline and 2443 ± 516 kcal/day at the 36-month follow-up).

To our knowledge (per personal communication with team head coaches), the physical activity of each athlete group, as described in the “participant characteristics” section in this chapter, remained consistent each of the 4 years of each athlete’s collegiate career.

Changes in aBMD

Table 3 shows the trend with age in aBMD at each skeletal site between and across groups. For total hip aBMD, the group-specific trend with age depends on group, which differed significantly by group. For the GYM and RUN groups, the trend is not significantly different from zero, whereas for controls it trends significantly ($p < 0.0001$) downward with age (table 3.3 and figure 3.1). GYM and RUN gained approximately 0.27% and 1.22% total hip aBMD, whereas CON lost 3.19% aBMD at the total hip over 36 months (table 3.4). For femoral neck aBMD, the estimate of the group-specific trend with age differs significantly across groups. For both the GYM and RUN groups, the trend is not significantly different from zero (femoral neck aBMD stays relatively flat), whereas the CON group trends significantly ($p < 0.0001$) downward with age (table 3.3 and figure 3.2). GYM, RUN, and CON experienced approximately 0.94%, 1.77%, 4.24% aBMD losses at the femoral neck, respectively (table 3.4). For trochanter aBMD, the trend with age does not depend on group, but a marginal trend with age (averaged across groups) is significantly different than zero. The trend for trochanter aBMD is also downward,

estimated to decrease by 0.00926 g/cm^2 per year ($p < 0.0001$) (table 3.3 and figure 3.3). GYM, RUN, and CON lost approximately 1.58%, 2.50%, and 3.94% trochanter aBMD, respectively over 36 months (table 3.4). Estimates of the group-specific trend with age for lumbar spine aBMD differed significantly by group. For the RUN and CON groups, the trend is not significantly different from zero, but the trend for the GYM group was significant ($p=0.0014$) and upward for lumbar spine aBMD (table 3.3 and figure 3.4). GYM and RUN gained approximately 2.70% and 1.12%, respectively, whereas CON lost about 0.06% lumbar spine aBMD over 36 months (table 3.4).

Discussion

Participation in sports during the childhood and adolescent years leads to high BMC gains, particularly in sports like artistic gymnastics that include maneuvers that generate high impact forces on the skeleton. The BMC gains achieved during the pubertal years appear to be sustained into young adulthood (4). Comparisons of areal bone mineral density (aBMD) in college-age athletes support the notion that athletes participating in sports that generate high impact ground reaction forces, have higher aBMD at most skeletal sites than their non-athletic counterparts. The question remains, does sports participation during the college years, a time associated with slow or no change in aBMD, augment aBMD and peak bone mass (PBM), or are the differences observed in young adulthood the result of childhood sports participation?

The current study is one of the few prospective reports comparing changes in aBMD in college athletes versus non-athletes from their freshman to senior year and the first study to look at the changes in aBMD over all four college years in gymnasts, runners, and controls. The primary finding of this study is that the two athlete groups [gymnasts (GYM) and runners (RUN)] showed no significant changes at the total hip or femoral neck aBMD 36 months, yet the

non-athlete control (CON) group had significant ($p < 0.0001$) declines at the total hip (-0.0106 g/cm^2) and femoral neck (-0.0129 g/cm^2). Furthermore, lumbar spine aBMD of the GYM increased significantly per year (0.0142 g/cm^2 , $p < 0.05$), but did not change in RUN or CON.

Consistent with our findings, two prospective studies reported significant increases at the lumbar spine: an 8-month prospective study of collegiate gymnasts, runners and controls found significantly greater lumbar spine aBMD gains in gymnasts (14) and a 3.6 year study of highly active white females observed 3.27% increase in lumbar spine aBMD (17). College-age adults thus may be able to augment vertebral aBMD if they participate in high-impact load activity. In contrast, gymnasts in our study did not gain hip aBMD, but sports participation did prevent losses. Gymnasts have shown hip gains at 8-months (14) and no change over 24 months (16), the later more consistent with our 36 month duration. Regardless, the controls in the current trial experienced significant losses at the total hip (3.2%) and femoral neck (4.2%) that were prevented with participation in gymnastics or running. The 2.26% increase reported at the hip by Drake et al is inconsistent with our study, however a control group was not included and compared to the highly active Naval cadets (17).

The sports training that occurred over the 36 months in the present study more than likely was the stimulus that led to the maintenance of total hip and femoral neck aBMD in the GYM and RUN and the lumbar spine aBMD gains seen in the GYM. It is possible, however, that differences in dietary intakes of calcium and vitamin D over the 36 months may have contributed to the differential bone response observed between athletes and non-athletes, but it is unlikely. Dietary information was not collected in this study. However, when dietary calcium and vitamin D are used as covariates in statistical models they do not seem to affect the relationship between physical activity and aBMD (18, 19). In college females, dietary calcium and vitamin D intakes

are not related to aBMD (20, 21), possibly due to the slower bone turnover during the young adult years. Typical calcium intakes in female athletes and non-athletes are similar. NCAA Division I cross-country runners report dietary calcium intakes of 605 mg/day (22) and collegiate gymnasts, 683 ± 58 mg/day (15). Similarly, calcium intakes in young adult, non-athletic females have been reported to be 647.2 ± 46.3 mg/day (23), 600 mg/day (24) and 753 ± 63 mg/day (15), below the current recommended adequate intake (AI) of 1000 mg/day of calcium for adult women over 19 years of age (25). Unless subjects have a calcium or vitamin D deficiency, diet does not seem to significantly affect aBMD when weight-bearing physical exercise is sufficient to provide mechanical stimuli (26-28).

Dietary supplement and ergogenic aid use is another variable not collected in this study, but one that could explain differences in our results vs. other studies, as 62% of female college students and 57-65% of female athletes report taking vitamin/mineral supplements regularly or sporadically (29, 30). Many athletes take a multi-vitamin supplement for ‘insurance’ purposes—to ensure that they are getting most of the nutrients they need—and in a study of 162 collegiate female varsity athletes, 60.1% cited “good health” as the reason for using supplements (31). The most common supplements used by athletes and the general college population are multi-vitamin/mineral supplements, vitamin C, and iron (29, 31). Other than maximizing health and performance, taking multi-vitamin/mineral supplements would increase calcium and vitamin D intakes to equal to or greater than the AI (23). Adequate intakes of calcium and vitamin D, made possible through multi-vitamin/mineral supplementation, may allow for optimal bone mineral gains in this age group. However, it has been proposed that physical activity has a greater effect on bone growth than diet—even when calcium and vitamin D intakes are sub-optimal (27). Also, supplementation trials in young adult athletes (32) and non-athletes (28) do not show

greater aBMD gains at the hip or spine in the supplemented group taking 1500 mg calcium/day (32). Due to the many lifestyle factors influencing bone it is important to consider that “nutrition alone does not influence muscle or bone in a dose-dependent manner” (33, 34).

Other factors that may have affected our findings are oral contraceptive (OC) use, age of menarche onset, and menstrual status. OCs could potentially affect our bone findings if one group had a higher prevalence of OC use than another group, but on average the prevalence of OC use between athlete groups and non-athletes varies. In recent studies, a higher percentage of athletes (40.2%) vs. controls (27.5%) reported OC use (35) whereas 33% versus 63% of gymnasts and non-athletes, respectively, reported using OCs (36). We did not collect information on OC use in this study; however, the potential benefits of OC use on aBMD are not clearly demonstrated. If the athlete groups in our study did have a higher prevalence of OC use, it may have either resulted in a protective effect on aBMD as was observed in a study of collegiate long distance runners and fracture rate (37) or no effect on aBMD (38-42). One of the most recent reviews of the literature by Martins, et al (43), from 1966-2005 on OC use in adolescents and young adult women concluded that research findings are still unclear as to whether OC use prevents young women (<23 years) from achieving their peak bone mass and that adult women who use OCs have similar aBMD to non-users.

Whereas the effect of OC use on bone in gymnasts and runners is still unclear and controversial, the adverse effects of delayed menarche and primary and secondary amenorrhea on bone are well documented (36, 44, 45). Menstrual dysfunction is more prevalent in athletes than non-athletes, with athletic amenorrhea being most common in long-distance runners and ballet dancers (46). In a study of 91 young adult competitive runners, abnormally menstruating runners (N=36) had lower aBMD than eumenorrheic runners (N=58) at the lumbar spine (-5%),

hip (-6%) and total body (-3%) after accounting for weight, % body fat, eating disorder inventory score, and age at menarche (47). Although it would have been beneficial to have information on the prevalence of amenorrhea in all groups, we only had menarchal status data for the CON group. Even if the athletic groups in the present study did have a higher prevalence of menstrual disturbances than controls, this study highlights the protective effect of high impact physical activity on bone.

In summary, we found that the non-athletic CON group had significant downward trends in total hip and femoral neck aBMD over 36 months. At the trochanter, the trend across all 3 groups was significant and downward, decreasing at 0.00926 g/cm^2 per year ($p < 0.0001$), whereas at the lumbar spine, the trend over time was significant and upward for GYM. Beyond gains in youth, it appears as though sports participation in the college years is advantageous to bone in young adulthood. Participating in sports during the college years may have a protective effect on aBMD at the total hip and femoral neck and a beneficial effect at the lumbar spine. The consensus that the total hip peaks around age 18-20 is consistent with the results from this study. Most interesting, however, is that according to our findings, aBMD losses begin during the college years—unless enough weight-bearing physical activity is performed.

Table 3.1.

Explanation for the difference in number of subjects from baseline to 36-month follow-up.

	Year 1 ¹	Year 2 ¹	Year 3 ¹	Year 4 ¹	Total
GYM					
N ⁵	37	29	20	16	
Quit ⁶	0	4	5	2	11
Lower classmen ⁷	0	4	4	2	10
RUN					
N ⁵	28	28	19	11	
Quit ⁶	0	0	4	1	5
Lower classmen ⁷	0	0	5	7	12

¹Freshman, Sophomore, Junior, Senior years, respectively

⁵Number of subjects per year

⁶Number of athletes who quit prior to that year's measurement

⁷Number of athletes who had not yet reached that year of college

Table 3.2. Participant characteristics at baseline & 36 month follow-up. ¹

	Artistic Gymnasts		Cross-Country Runners		Control Subjects	
	N=37	N=16	N=28	N=11	N=70	
	Baseline	36 month	Baseline	36 month	Baseline	36 month
Age & Anthropometry						
Age (yr)	18.5±0.4	21.6±0.3	18.8 ±0.6	21.6±0.4	18.7±0.3	21.8±0.3
Ht (in)	62.3±2.1	62.6±1.7	65.5±2.1	64.9±2.6	65.1±2.4	65.2±2.4
Wt (lbs)	123.2±14.2	128.2±10.9	123.0±12.1	120.8±10.9	132.5±17.6	135.2±21.9
BMI	22.3±2.0	23.0±2.0	20.3±1.9	20.1±1.8	22.0±2.6	22.4±3.2
Body Composition²						
Fat Mass (kg)	11.4±2.5	12.0±2.0	11.9±2.6	11.8±3.6	17.5±4.7	18.7±6.3
Lean Mass (kg)	43.8±48.4	45.2±3.6	42.7±3.7	42.0±2.1	41.6±4.5	41.9±4.8
Body Fat %	19.7±3.1	20.1±2.2	20.8±3.1	20.8±4.6	28.3±4.2	29.2±5.0
Total Body²						
BA (cm ²)	1937±138.9	2004±114.8	1938±137.0	1929±149.3	1947±165.4	1973±170.6
BMC (g)	2222±266.3	2328±212.9	2117±233.0	2121±197.8	2053±294.8	2098±303.1
aBMD (g/cm ²)	1.145±0.085	1.161±0.068	1.091±0.071	1.101±0.052	1.050±0.081	1.060±0.080
Femoral Neck (FN)²						
BA (cm ²)	3.897±0.723	4.087±0.638	4.607±0.367	4.713±0.247	4.443±0.431	4.620±0.452
BMC (g)	4.26±0.89	4.54±0.86	4.20±0.49	4.05±0.48	4.04±0.66	4.03±0.67
aBMD (g/cm ²)	1.096±0.144	1.116±0.087	0.914±0.094	0.888±0.104	0.911±0.124	0.871±0.114
Trochanter (TR)²						
BA (cm ²)	9.77±0.93	10.18±1.02	10.46±0.98	10.19±1.10	10.24±2.02	10.36±1.50
BMC (g)	8.81±1.50	9.02±1.26	8.18±1.25	7.39±0.92	7.83±2.79	7.49±1.82
aBMD (g/cm ²)	0.901±0.118	0.890±0.094	0.781±0.092	0.763±0.087	0.750±0.121	0.717±0.099
Total Hip²						
BA (cm ²)	31.0±4.4	32.0±2.1	33.3±2.8	32.6±3.2	31.6±3.5	32.2±3.0
BMC (g)	35.1±5.7	36.4±4.1	33.2±3.9	32.0±3.1	31.1±5.7	30.6±5.0
aBMD (g/cm ²)	1.135±0.117	1.136±0.089	0.999±0.100	1.001±0.100	0.982±0.113	0.949±0.102

Lumbar Spine ²						
BA (cm ²)	54.7±5.0	57.0±4.8	58.9±5.0	58.1±5.5	57.7±5.7	58.1±6.1
BMC (g)	61.8±10.3	65.1±7.2	56.5±9.2	56.2±8.0	57.8±9.7	58.2±10.2
aBMD (g/cm ²)	1.125±0.115	1.140±0.070	0.955±0.104	0.960±0.060	0.999±0.107	1.000±0.100

¹All values are mean ± standard deviation.

Baseline was measured in August of freshman year and 36-month follow-up measured in August of senior year of college.

²Measurements assessed using DXA

Table 3.3. Areal BMD (g/cm²) trend with age between and across groups

	GYM N=37	RUN N=28	CON N=70	ALL
Total Hip (TH)	NS	NS	(-)0.0106 [*]	NS
Femoral Neck (FN)	NS	NS	(-)0.0129 [*]	NS
Trochanter (TR)	NS	NS	NS	(-)0.00926 [*]
Lumbar Spine (LS)	(+)0.5142 [§]	NS	NS	NS

[§]p<0.05

^{*}p<0.0001

NS = not significant (p>0.05)

Table 3.4. Mean percent change over 36 months¹

	GYM N=16	RUN N=11	CON N=70	P-value	
Age & Anthropometry					
Wt (lbs)	1.06±7.16	2.12±5.86	2.13±8.96	0.899	
BMI	1.18±7.46	1.16±5.92	1.64±8.89	0.971	
Body Composition ²					
Fat Mass (g)	5.62±20.87	7.67±20.43	7.75±24.58	0.948	
Lean Mass (kg)	0.1965±5.78	2.12±3.56	0.75±4.95	0.602	
Body Fat %	3.81±14.19	3.46±13.86	4.02±14.37	0.992	
Total Body ²					
BA (cm ²)	1.47±2.91	2.99±3.37	1.34±1.83	0.097	
BMC (g)	3.31±5.38	4.09±3.78	2.22±2.66	0.159	
aBMD (g/cm ²)	1.88±4.07	0.85±1.86	0.89±2.42	0.405	
Femoral Neck (FN) ²					
BA (cm ²)	7.06±19.77	3.69±3.55	4.28±6.9	0.567	
BMC (g)	5.77±19.41	1.8±5.17	(-)0.19±6.94	0.097	
aBMD (g/cm ²)	(-)0.94±6.69	(-)1.77±5.03	(-)4.24±4.05	0.021	GYM>CON
Trochanter (TR) ²					
BA (cm ²)	2.1±5.45	0.52±7.54	3.02±14.39	0.822	
BMC (g)	0.52±9.57	(-)1.8±11.97	(-)0.72±16.24	0.921	
aBMD (g/cm ²)	(-)1.58±7.24	(-)2.5±6.89	(-)3.94±4.68	0.254	
Total Hip ²					
BA (cm ²)	5.79±6.56	1.35±4.68	2.38±7.18	0.156	
BMC (g)	5.97±6.74	2.52±5.84	(-)0.84±8.35	0.007	GYM>CON
aBMD (g/cm ²)	0.27±5.34	1.22±4.87	(-)3.19±3.56	0.000	GYM, RUN>CON
Lumbar Spine ²					
BA (cm ²)	2.92±4.29	(-)0.21±3.73	0.81±5.13	0.201	
BMC (g)	5.8±8.70	1.01±6.77	0.84±7.59	0.069	GYM>CON
aBMD (g/cm ²)	2.7±5.59	1.12±3.64	(-)0.06±4.1	0.068	GYM>CON

¹All values are mean ± standard deviation.

Baseline was measured freshman year and 36-month follow-up was measured senior year of college.

²Measurements assessed using DXA

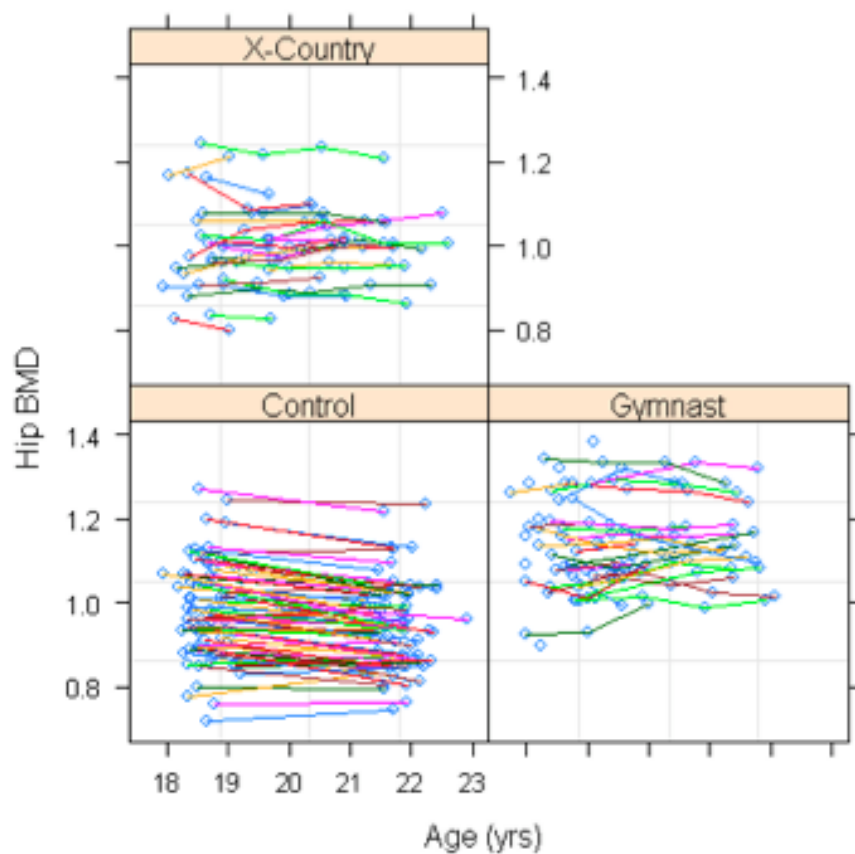


Figure 50. Total hip aBMD (g/cm^2) changes over 36 months in GYM, RUN, and CON.

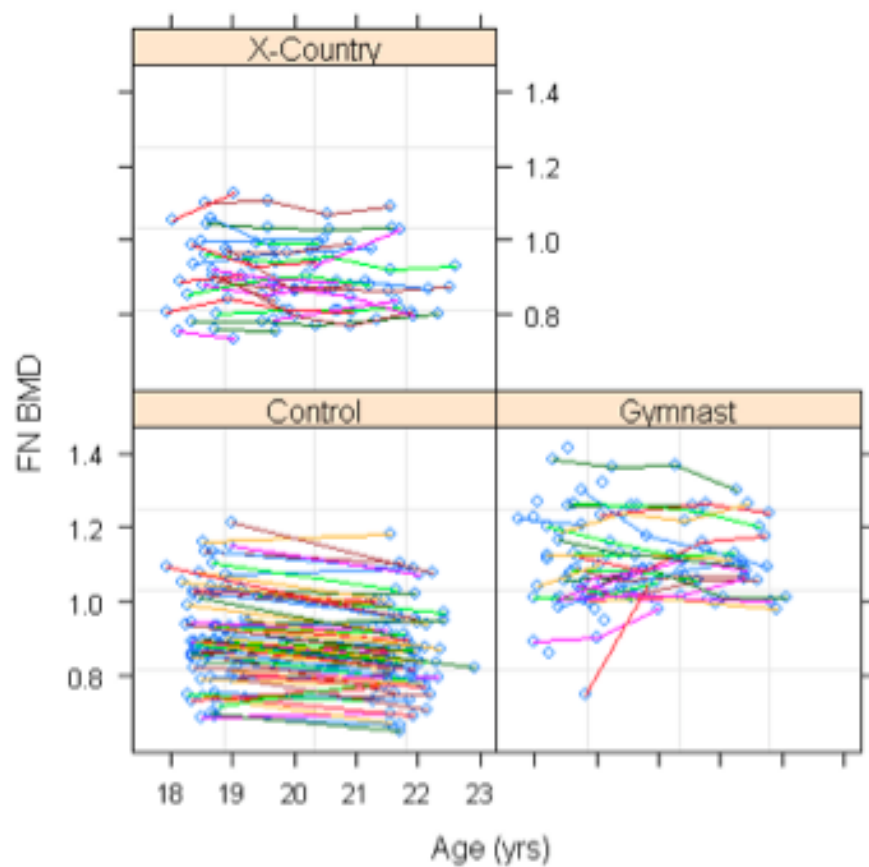


Figure 50. Femoral neck aBMD (g/cm^2) changes over 36 months in GYM, RUN and CON.

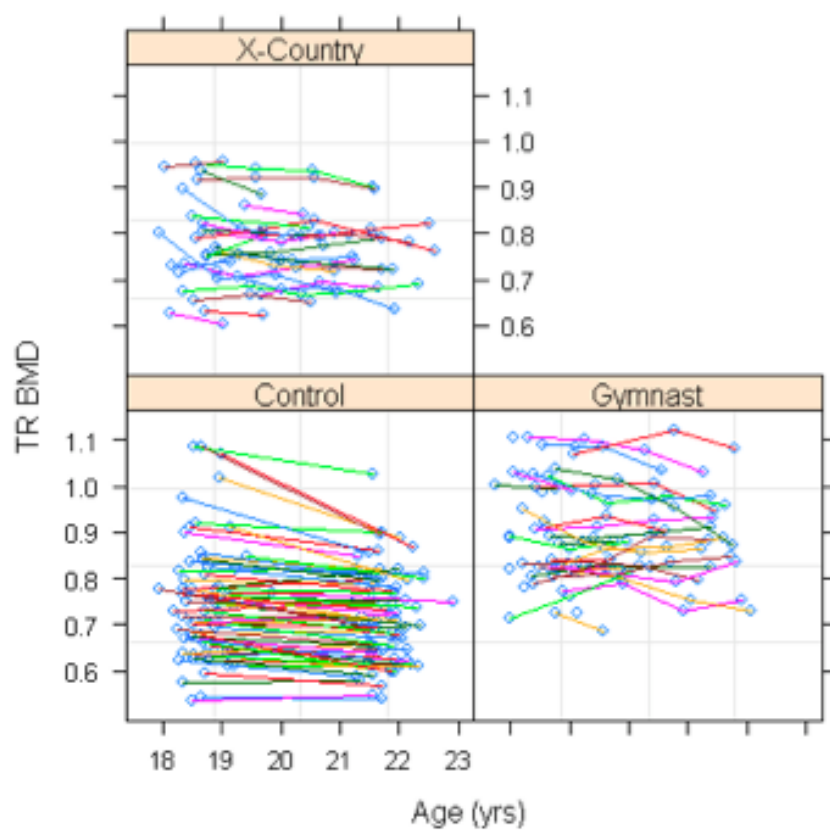


Figure 5B. Trochanter aBMD (g/cm²) changes over 36 months in GYM, RUN and CON.

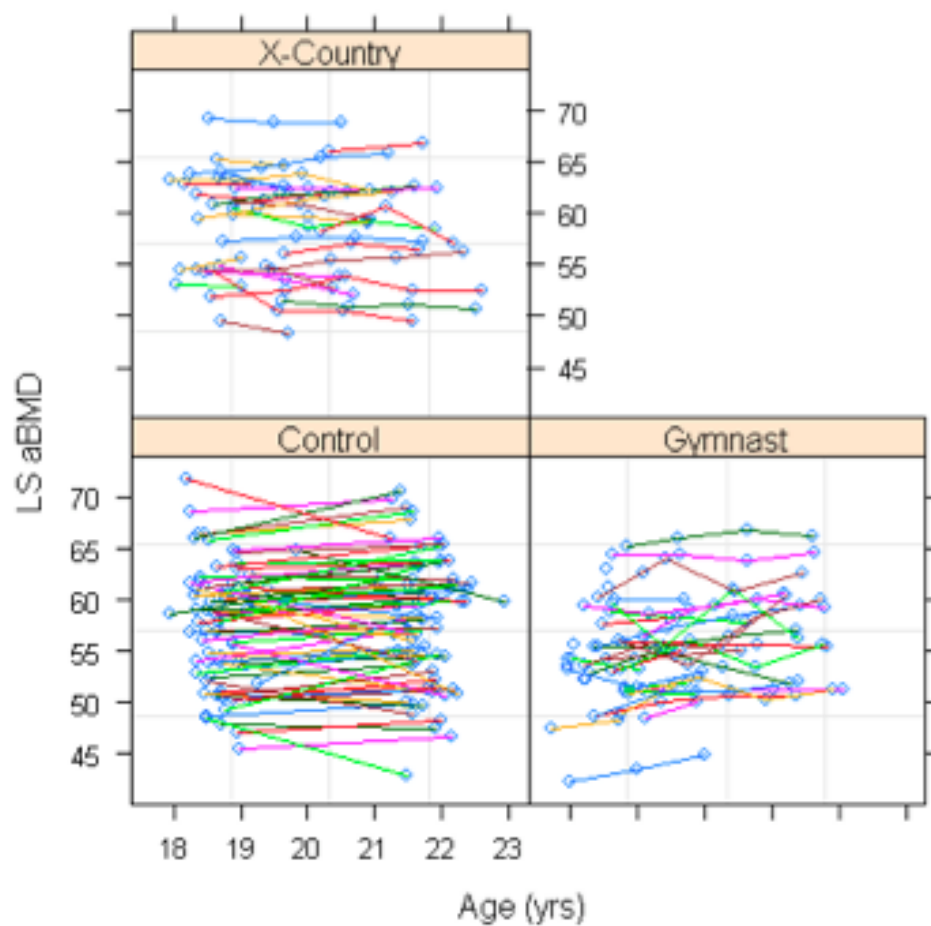


Figure 3.4. Lumbar spine aBMD (g/cm^2) changes over 36 months in GYM, RUN and CON.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

The benefits of high-impact physical activity on bone throughout the life cycle are well documented. In children, jumping and exercise intervention trials, as well as cross-sectional (1) and longitudinal (2, 3) studies of gymnasts have been instrumental in documenting the benefits of impact-loading activities on growing bone. More recently it has been shown that the bone mineral gained during childhood and adolescence are maintained into young adulthood (4, 5). In young adults, cross-sectional studies of athletes in various sports also show the associations of high-impact-loading versus non-weight-bearing activities on bone strength (6-10). Comparisons of areal bone mineral density (aBMD) in college-age athletes support the notion that athletes participating in sports that generate high-impact ground-reaction forces have higher aBMD at most skeletal sites than their non-athletic counterparts. The question remains, does sports participation during the college years, a time associated with slow or no change in aBMD, augment aBMD and peak bone mass (PBM), or are the differences observed in young adulthood the result of childhood sports participation?

The current study is one of the few prospective reports comparing changes in aBMD in college athletes versus non-athletes from their freshman to senior year and the first study to look at the changes in aBMD over all four college years in gymnasts, runners, and controls. The primary finding of this study is that the two athlete groups [gymnasts (GYM) and runners (RUN)] showed no significant changes at the total hip or femoral neck aBMD over 36 months, yet the non-athlete control (CON) group had significant ($p < 0.0001$) declines at the total hip (-

0.0106 g/cm²) and femoral neck (-0.0129 g/cm²). Furthermore, lumbar spine aBMD of the GYM increased significantly per year (0.5142 g/cm², $p < 0.05$), but did not change in RUN or CON.

Consistent with our findings, two prospective studies reported significant increases at the lumbar spine: an 8 month prospective study of collegiate gymnasts, runners and controls found significantly greater lumbar spine aBMD gains in gymnasts (11) and a 3.6 year study of highly active white females observed 3.27% increase in lumbar spine aBMD (12). College-age adults thus may be able to augment vertebral aBMD if they participate in high-impact load activity. In contrast, gymnasts in our study did not gain hip aBMD, but sports participation did prevent losses. Gymnasts have shown hip gains at 8 months (11) and no change over 24 months (13), the latter more consistent with our 36 month duration. Regardless, the controls in the current trial experienced significant losses at the total hip (3.2%) and femoral neck (4.2%) that were prevented with participation in gymnastics or running. The 2.26% increase reported at the hip by Drake et al is inconsistent with our study, however a control group was not included and compared to the highly active Naval cadets (12).

The sports training that occurred over the 36 months in the present study more than likely was the stimulus that led to the maintenance of total hip and femoral neck aBMD in the GYM and RUN and the lumbar spine aBMD gains seen in the GYM. It is possible, however, that differences in dietary intakes of calcium and vitamin D over the 36 months may have contributed to the differential bone response observed between athletes and non-athletes, but it is unlikely. Dietary information was not collected in this study. However, when dietary calcium and vitamin D are used as covariates in statistical models they do not seem to affect the relationship between physical activity and aBMD (14, 15). In college females, dietary calcium and vitamin D intakes are not related to aBMD (16, 17), possibly due to the slower bone turnover during the young

adult years. Typical calcium intakes in female athletes and non-athletes are similar. NCAA Division I cross-country runners report dietary calcium intakes of 605 mg/day (18) and collegiate gymnasts, 683 ± 58 mg/day (19). Similarly, calcium intakes in young adult, non-athletic females have been reported to be 647.2 ± 46.3 mg/day (20), 600 mg/day (21) and 753 ± 63 mg/day (19), below the current recommended adequate intake (AI) of 1000 mg/day of calcium for adult women over 19 years of age (22). Unless subjects have a calcium or vitamin D deficiency, diet does not seem to significantly affect aBMD when weight-bearing physical exercise is sufficient to provide mechanical stimuli (23-25).

Dietary supplement and ergogenic aid use is another variable not collected in this study, but one that could explain differences in our results vs. other studies, as 62% of female college students and 57-65% of female athletes report taking vitamin/mineral supplements regularly or sporadically (26, 27). Many athletes take a multi-vitamin supplement for ‘insurance’ purposes—to ensure that they are getting most of the nutrients they need—and in a study of 162 collegiate female varsity athletes, 60.1% cited “good health” as the reason for using supplements (28). The most common supplements used by athletes and the general college population are multi-vitamin/mineral supplements, vitamin C, and iron (26, 28). Other than maximizing health and performance, taking multi-vitamin/mineral supplements would increase calcium and vitamin D intakes to equal to or greater than the AI (20). Adequate intakes of calcium and vitamin D, made possible through multi-vitamin/mineral supplementation, may allow for optimal bone mineral gains in this age group; however, it has been proposed that physical activity has a greater effect on bone growth than diet—even when calcium and vitamin D intakes are sub-optimal (24). Also, supplementation trials in young adult athletes (29) and non-athletes (25) do not show greater aBMD gains at the hip or spine in the supplemented group taking 1500 mg calcium/day

(29). Due to the many lifestyle factors influencing bone it is important to consider that “nutrition alone does not influence muscle or bone in a dose-dependent manner” (30, 31).

Other factors that may have impacted our findings are oral contraceptive (OC) use, age of menarche onset, and menstrual status. OCs could potentially affect our bone findings if one group had a higher prevalence of OC use than another group, but on average the prevalence of OC use between athlete groups and non-athletes is mixed. In recent studies, a higher percentage of athletes (40.2%) vs. controls (27.5%) reported OC use (32) whereas 33% versus 63% of gymnasts and non-athletes, respectively, reported using OCs (33). If the athlete groups in our study did have a higher prevalence of OC use, it may have either resulted in a protective effect on aBMD as was observed in a study of collegiate long distance runners and fracture rate (34) or no effect on aBMD (35-39). One of the most recent reviews of the literature by Martins, et al (40), from 1966-2005 on OC use in adolescents and young adult women concluded that research findings are still unclear as to whether OC use prevents young women (<23 years) from achieving their peak bone mass and that adult women who use OCs have similar aBMD to non-users.

Whereas the effect of OC use on bone in gymnasts and runners is still unclear and controversial, the adverse effects of delayed menarche and primary and secondary amenorrhea on bone are well documented (33, 41, 42). Menstrual dysfunction is more prevalent in athletes than non-athletes with athletic amenorrhea being most common in long-distance runners and ballet dancers (43). In a study of 91 young adult competitive runners, abnormally menstruating runners (n=36) had lower aBMD than eumenorrheic runners (n=58) at the lumbar spine (-5%), hip (-6%) and total body (-3%) after accounting for weight, % body fat, eating disorder inventory score, and age at menarche (44). Although it would have been beneficial to have information on

the prevalence of amenorrhea in all groups, we only had menarchal status data for the CON group. Even if the athletic groups in the present study did have a higher prevalence of menstrual disturbances than controls, this study highlights the protective effect of high impact physical activity on bone.

In summary, we found that the non-athletic CON group had significant downward trends in total hip and femoral neck aBMD over 36 months. At the trochanter, the trend across all 3 groups was significant and downward, decreasing at 0.00926 g/cm^2 per year ($p < 0.0001$), whereas at the lumbar spine, the trend over time was significant and upward for GYM. Beyond gains in youth, it appears as though sports participation in the college years is advantageous to bone in young adulthood. Participating in sports during the college years may have a protective effect on aBMD at the total hip and femoral neck and a beneficial effect at the lumbar spine. The consensus that the total hip peaks around age 18-20 is consistent with the results from this study. Most interesting, however, is that according to our findings, aBMD losses begin during the college years—unless enough weight-bearing physical activity is performed.

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APPENDICES

APPENDIX A

Consent Forms

UGA Bone and Body Composition Lab

Consent Form for the Use of the Hologic Delphi A X-Ray Bone Densitometer

Are you pregnant or do you think you might be pregnant? YES NO

*If yes, please do not participate in this study using the Delphi A bone densitometer.

I, _____, am hereby giving my consent to be used for research conducted by Dr. Richard D. Lewis, University of Georgia, Foods and Nutrition Department, 279 Dawson Hall.

I understand that by giving my consent I am agreeing to be scanned on the Hologic Bone Delphi A Densitometer machine. This instrument uses a low dose X-ray to determine bone mineral density and body composition.

I understand that the Hologic Delphi A Bone Densitometer uses a very low level of X-ray and that under most operating conditions, the entrance dose to the patient is 0.5mRem-10mRem. This equals about 3% to 30% of the exposure of a standard chest X-ray and is of no danger to me.

I understand that The University of Georgia is responsible for my safety during my participation in this study. However, any illness or injury not related to this study is not the responsibility of the investigator or The University of Georgia.

I understand that my participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of my participation returned to me, removed from records or destroyed.

Signature of Investigator Date

Signature of Participant Date

Soy, Bone, and Health in College Females: A Follow-Up
Verbal consent script

Good Afternoon. My name is _____ from the University of Georgia Department of Foods and Nutrition. I am calling today to ask you a few questions to determine your eligibility for participation in a research study entitled “Soy, Bone, and Health in College Females: A Follow-Up”. This interview should only take approximately ten minutes.

The purpose of this study is to determine if body composition may have implications in bone structure and strength during the college years.

To qualify for the study, you must:

- Have participated in the “Soy, Bone, and Health in College Females” in 2005
- Agree to come to the Bone and Body Composition Lab at the University of Georgia for blood work, bone density testing and to complete several questionnaires (once during your junior year and once during your senior year)
- Not be pregnant or have intentions of becoming pregnant during the study
- Answer questions over the phone regarding your age, body size, physical activity level, and brief medical history including your medication use and menstrual status
- Provide written consent to the procedures described below, following an overnight fast
 - A consent form will be provided to you (either via email or postal mail) for your review prior to enrolling in the study.
- During your visit, the following procedures will be done:
 - Weight, height and vital signs will be taken
 - A fasting blood sample will be taken from an arm vein;
 - Bone density will be measured with two bone-scanning machines;
 - Completion of the following questionnaires:
 - A Health History Questionnaire
 - A Dietary Questionnaire
 - A Physical Activity Questionnaire
- You will receive \$35 if you complete the study.
- Reasonably foreseeable risks or discomforts from participating in this study are minimal, but may include:
 - Hypoglycemia, or low blood sugar, resulting from fasting when providing written consent (some symptoms of hypoglycemia include: trembling, clamminess, palpitations, anxiety, sweating, hunger, difficulty in thinking, and confusion).
 - An evaluation of your food intake, physical activity, body weight fluctuations, and personal/family medical history.

Do you have any questions?

Let me assure you that any information you provide will be kept strictly confidential. Your participation in providing me with information on previous and current health information is completely voluntary and you may refuse to participate or discontinue our interaction at any time or skip any question you do not wish to answer without penalty or loss of benefits to which you are otherwise entitled. If you are determined to be ineligible for the study, the screening data collected over the telephone will be immediately destroyed.

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu

Are you interested in participating in this study? Do you verbally agree to participate?

[If yes, continue to telephone screen]

[If not, say "Thank you for your time, goodbye."]

Provide researcher's contact information at the end of the interaction.

Richard D. Lewis

279 Dawson Hall

The University of Georgia

706-542-4901, rlewis@fcs.uga.edu

APPENDIX B

Physical Activity Questionnaires

Subject Code No. _____

Date _____

7-DAY PHYSICAL ACTIVITY RECALL QUESTIONNAIRE

1. On the average, how many hours did you sleep each night during the last 5 weekday nights (Sunday-Thursday)? Record to nearest quarter-hour.

Hours: _____ Minutes: _____

2. On the average, how many hours did you sleep each night last Friday and Saturday nights?

Hours: _____ Minutes: _____

3. First let's consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour.

Hours: _____ Minutes: _____

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

Hours: _____ Minutes: _____

5. Now let's look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.

Hours: _____ Minutes: _____

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

Hours: _____ Minutes: _____

7. Now let's look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

Hours: _____ Minutes: _____

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Can you think of other sport, job, or household activities that would fit in this category?)

Hours: _____ Minutes: _____

Physical Activity List

Moderate Activities

Occupational Tasks:

9. Delivering mail or patrolling on foot
10. House painting
11. Truck driving (making deliveries – lifting and carrying light objects)

Household activities:

1. Raking the lawn
2. Sweeping and mopping
3. Mowing the lawn with a power mower
4. Cleaning windows

Sports Activities (Actual playing time)

1. Volleyball
2. Ping pong
3. Brisk walking for pleasure or to work (3 mph or 20 min/mile)
4. Golf-walking and pulling or carrying clubs
5. Calisthenic exercises

Hard Activities

Occupational Tasks:

1. Heavy carpentry
2. Construction work – doing physical labor

Household Tasks:

1. Scrubbing floors

Sports Activities (Actual playing time):

1. Doubles tennis
2. Disco, Square, or Folk dancing

Very Hard Activity

Occupational Tasks:

1. Very Hard physical labor – digging or chopping with heavy tools
2. Carrying heavy loads, such as bricks or lumber

Sports Activities (Actual playing time):

- | | |
|------------------------|--------------------|
| 1. Jogging or swimming | 5. Aerobics |
| 2. Singles tennis | 6. Stair climbing |
| 3. Racquetball | 7. Weight training |
| 4. Soccer | 8. Gymnastics |

7-DAY PHYSICAL ACTIVITY RECALL

	Activity	Time Spent
1.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
2.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
3.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
4.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
5.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
6.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
7.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>
8.	<hr/> <hr/> <hr/>	<hr/> <hr/> <hr/>

 Worksheet for Calculating Daily Energy Expenditure

- | | | |
|-----|---|-----------------------------|
| 1. | Add up all the hours of sleep and naps you had. | |
| 2. | Multiply the total number of hours of sleep and naps (line 1) by 1. | <u> </u> |
| | | <u> X 1 = </u> |
| 3. | Add up the total number of hours spent in moderate activity. | <u> </u> |
| 4. | Multiply the hours spent in moderate activity (line 3) by 4. | <u> X 4 = </u> |
| 5. | Add up the total number of hours spent in hard activity. | <u> </u> |
| 6. | Multiply the hours spend in hard activity (line 5) by 6. | <u> X 6 = </u> |
| 7. | Add up the total number of hours spent in very hard activity. | <u> </u> |
| 8. | Multiply the hours spent in very hard activity (line 7) by 10. | <u> X 10 = </u> |
| 9. | Add up the figures in lines 1, 3, 5, and 7.
(1 + 3 + 5 + 7) = | <u> </u> |
| 10. | Hours spent in light activity is equal to 24 hours minus the hours in
lines 1, 3, 5, and 7.
24 - (1 + 3 + 5 + 7) = | <u> </u> |
| 11. | Multiply the figure in line 10 by 1.5. | <u> X 1.5 = </u> |
| 12. | Add up the figures in lines 2, 4, 6, 8, and 11.
(2 + 4 + 6 + 8 + 11) = | <u> </u> |
| 13. | The figure you arrived at in line 12 is the total kilocalories per
kilogram of body weight expended per day.
(kcal • kg ⁻¹ • day ⁻¹) = | <u> </u> |
| 14. | To calculate the total number of calories you expended in one day,
multiply your total body weight in kilograms)weight in pounds ÷
2.2046 = kilograms) by the figure in line 13. Body weight (kg) X
kcal • kg ⁻¹ • day ⁻¹ = total calories expended = | <u> </u> |
-

The following are some average kcal • kg⁻¹ • day⁻¹ for individuals of different ages:

17-19 years
male = 44
female = 35

20-29 years
male = 40
female = 35

30-39 years
male = 38
female = 33

40-49 years
male = 37
female = 31

50-59 years
male = 36
female = 30

60-69 years
male = 34
female = 29

APPENDIX C

Health History Questionnaire

Soy, Bone, and Health in College Females: A Follow-Up

Health History Questionnaire

Subject ID# _____
 Interviewer _____
 Date _____

Surgery/Medication/Fracture History

1. Please list major medical procedures, surgeries and/or injuries in your lifetime and related medications. Give the time of the procedure or injury and/or the frequency and duration of medication.

2. Have you ever gone through an extended period of time where you were bedridden or immobilized?
 YES or NO; *circle one*
 - If yes, how old were you and how long did this immobilization last?
 - Briefly explain the circumstances.

3. Are you currently taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? YES or NO; *circle one*
 - If yes, what medications?

4. Has any member of your family been diagnosed with any medical condition related to obesity or osteoporosis? YES or NO; *circle one*

5. Have you ever experienced a skeletal fracture in your lifetime? YES or NO; *circle one*
 - If yes, at what age did you experience a fracture?
 - In what type of circumstance did the fracture take place?
 - How was the fracture treated (casting, medication, rest, etc.)?

Other History

1. How would you rate your present health? ____Poor____Good____Fair____Excellent
2. Do you currently smoke cigarettes? _ YES or NO; *circle one*
 - a. If yes, on the average, about how many cigarettes a day do you smoke?
____1-5, ____6-14, ____15-24, ____25-35, ____35 or more
3. If you used to smoke but do not smoke now, how long did you smoke? ____years.
4. At what age did you start your menstrual cycles? _____
5. Are your menstrual cycles regular? YES or NO; *circle one*
 - a. If not, how long have they been irregular? _____
6. Have you ever used birth control pills? YES or NO; *circle one*
 - a. How old were you when you began using birth control pills? _____
 - b. How long have you been using them? _____
7. What periods of time did you stop using birth control pills? (Please give dates, if applicable)
8. Are you on any nutritional supplements? _____
9. Are you currently dieting, or on a special type of weight loss program? YES or NO; *circle one*
 - a. If yes, what program are you following? _____
10. Do you have any health problems that limit your physical activity? _____

How many hours, on average, do you spend watching TV, or on the computer? _____