THE EFFECT OF PHYSICAL ACTIVITY PARTICIPATION DURING ADOLESCENCE ON CURRENT BMD IN PREMENOPAUSAL FEMALES 30-50 YEARS OLD

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Abstract

THE EFFECT PHYSICAL ACTIVITY PARTICIPATION DURING ADOLESCENCE ON CURRENT BMD IN PREMENOPAUSAL FEMALES 30-50 YEARS OLD

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Physical activity (PA) and nutrition are essential for bone health. Low bone mineral density is often a concern with women and may lead to major health concerns such as increased fracture risk and osteoporosis. PURPOSE: To examine the relationship between past and current nutrition and physical activity with current bone mineral density (BMD) in order to determine what affect they have on bone health. METHODS: Pre-menopausal females ages 30-50 years (N=27) completed a DEXA scan to determine total BMD and answered a series of surveys concerning basic demographics and previous and current physical activity participation and calcium consumption. Questions included information related to menstrual cycle, bone health, and sport participation. Previous physical activity participation (PA between ages 14-21 years) provided total and average hours per year. Current moderate and vigorous intensity PA was also determined. Previous (during ages 13-18 years) diet recall provided frequency of consumption of calcium rich foods. A three-day diet diary provided current calcium intake as a percentage of recommended value. RESULTS: There was a correlation of 0.37 between PA ages 14-21 years and current BMD.

Dairy consumption ages 13-18 years showed a correlation of 0.28 with current BMD. Current time in moderate and vigorous intensity PA had a 0.23 correlation with current BMD. Current calcium intake showed a correlation of 0.17 with current BMD. Three females had a low Z-score (Z-score < -1.0) (Nattiv et al., 2007) and showed lower levels of PA during adolescence and currently. CONCLUSIONS: There is evidence of a relationship between previous and current PA with current BMD in females aged 30-50 years; however previous PA did not show as strong a correlation as previous studies. Nonetheless, calcium intake (especially during adolescence) was correlated with current BMD in females aged 30-50 years, suggesting both PA and diet are important to bone health.

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Chapter 1: Introduction and Literature Review

Introduction

Nutrition and physical activity are important for bone health and development. Adequate nutrition provides essential vitamins and minerals, which enable the bones to grow and become stronger. Physical activity (PA) further promotes bone health as stresses placed on bones stimulate adaptations that ultimately encourage the development of stronger bones in order to support optimal bone health.

A critical period for bone development is adolescence when bones are growing in both length and width With PA being important for optimal bone development, it is a concern that PA levels tend to plateau or even decline during adolescence, particularly in girls. This lack of physical activity has the potential to play a role in overall bone development that may not be noticed until later in adulthood, especially after menopause (Vatanparast, Bailey, Baxter-Jones, & Whiting, 2010).

Bone health is often reflected in bone mineral density (BMD). A healthy bone would be one that is dense, meaning it can withstand forces applied to it without breaking. A dense, healthier bone would have normal to high BMD, whereas a bone with low BMD is more prone to fracturing. Low bone mineral density is more of a concern in females compared to males and is typically examined in postmenopausal females due to bones' relationship with estrogen. The concern with low BMD is that it may lead to a reduced quality of life by increasing the risk of bone fractures and immobility. It is important to have a good diet and to participate in life long physical activity in order to maintain bone health.

Diseases associated with bone include osteoporosis and osteopenia. Osteoporosis is a disease where the bone becomes brittle and susceptible to fracture. It is typically seen in post-menopausal females due to the lower levels of estrogen (Nattiv et al., 2007). Estrogen is important since it is known to stimulate bone building cells or osteoblasts. Osteopenia often is the start of osteoporosis and may lead to an increased loss of bone mineral. Osteopenia is defined as a BMD with a Z score below -1.0 while osteoporosis is a Z score below -2.5 (Nattiv et al., 2007); therefore, females with BMD < -1.0 would be considered to be at risk for further bone loss and perhaps osteoporosis. The health concern is that post-menopausal females are at risk of developing osteoporosis. In fact, statistics from the National Health and Nutrition Examination Survey (NHANES) state 49% of females over age 50 years have osteopenia (Looker, Melton, Harris, Borrud, & Shepherd, 2010). Thus, it is important to study BMD and find a way to prevent osteoporosis.

Low BMD is often related to an accelerated bone mineral loss as an adult or inadequate bone development during childhood and adolescence (Nattiv et al., 2007). To avoid low BMD, healthy bone practices are important. Such practices include physical activity and good nutrition that promote bone health and provide optimal bone mineral density ultimately strengthening bone and reducing the risk of osteopenia, osteoporosis, and thus fractures. Participating in physical activity and eating a well-balanced diet may occur during adolescence when bones are growing in length and width and bone development is essential for one's future bone health. This bone development includes increases in length of long bones causing increased height, increases in BMD and bone mass area, and the transformation of woven bone to lamellar bone (both cortical and trabecular). During adolescence, peak bone mass must be reached to lower the future risk of developing osteoporosis (Vatanparast et al., 2010). Peak bone mass is typically achieved at or near maturation, suggesting proper nutrition and participation in physical activity is important during adolescence.

Physical activity is important for bone health. Weight bearing physical activity is important throughout life for achieving and maintaining bone mineral density (Greer & Krebs, 2006); however, it has been suggested that the most critical periods are in childhood and adolescence (Bailey, McKay, Mirwald, Crocker, & Faulkner, 1999; Gunter, Almstedt, & Janz, 2012; Kannus et al., 1995). What occurs during this time period may have long lasting effects on BMD (Bailey et al., 1999). For example, physical activity during preadolescence increases the density and size of cortical bone and the density of trabecular bone (Michalopoulou et al., 2013). Peak calcium-accretion rate is attained at an average age of 12.5 years in girls and 14.0 years in boys, when adult height is normally achieved (Bailey, Martin, McKay, Whiting, & Mirwald, 2000; Bailey et al., 1999; Greer & Krebs, 2006). Bone absorbs most of its calcium during adolescence and maturation (around 12.5 and 14 years respectively girls and boys) in which 40% of total bone mass is accumulated (Greer & Krebs, 2006). Therefore, the importance of weight bearing physical activity during adolescence should be emphasized.

Physical activity and good nutrition are important for bone health (Bailey et al., 1999; Nattiv et al., 2007; Vatanparast et al., 2010); however there is debate concerning which is more important. For example, regular weight bearing activity was shown to have a greater influence on BMD in comparison to calcium intake in children (Greer & Krebs, 2006), yet, nutrition is also important, specifically calcium and vitamin D. In fact, 99% of calcium in the body is found in bone and its main role is improving and sustaining bone mineral density.

Thus a diet low in calcium impacts bone and increases the risk for bone fractures (Greer & Krebs, 2006). Main sources of calcium include dairy (e.g., milk, yogurt, cheese, calcium fortified soymilk) as well as cereal and fruit juice (Greer & Krebs, 2006). The recommended daily calcium intake is 1300 mg per day in ages 9-18 years of age while adult females (19-50 years) should consume 1000 mg calcium each day (Greer & Krebs, 2006).

A diet composed of recommended calories and vitamins and minerals is important to bone health. An adequate intake of calcium during the growth period is essential for maximizing peak bone mineral density (Vatanparast et al., 2010). Adequate vitamin D intake is also important for optimal bone growth and peak bone mass (Koo & Walyat, 2013). Additionally, energy intake and availability needs to be sufficient for bone growth to allow an increasing BMD during the growth period (Greer & Krebs, 2006). Thus, diet plays an important role in the overall bone health, specifically during adolescence.

A current public health concern specific to active females is the Female Athlete Triad. The Triad consists of three inter-related components: osteoporosis, amenorrhea, and low energy availability (Nattiv et al., 2007). The Triad and combinations of its components have been reported in both elite athletes as well as in recreationally active females. Low energy availability, often a result of restricted caloric intake or extensive exercise, may lead to nutritional deficiencies, including low calcium intake. Energy availability is defined as energy intake minus exercise energy expenditure per fat-free mass or how much energy remains for body functions. Energy availability should be at least 30 kcal / kg fat-free mass for females in order for most body functions to work adequately (Nattiv et al., 2007). Additionally, a negative energy balance may lead to more serious complications such as interfering with the menstrual cycle. The menstrual cycle is an important body function and bone health is affected by the maintenance of a normal menstrual cycle and dependent upon associated hormones, including estrogen. If energy intake is not sufficient, estrogen levels are decreased, which in turn impacts BMD by an increased calcium resorption (Mallinson, Williams, Hill, & De Souza, 2013). Low energy availability is linked to changes in luteinizing hormone (LH), a main hormone in the regulation of the menstrual cycle. Specifically, LH pulsatility is disrupted (decreased LH pulse frequency and increased LH pulse amplitude) when the energy balance is significantly negative (Loucks & Thuma, 2003).

An alteration in menstrual function also suggests hormones related to the menstrual cycle are low, such as estrogen. Low levels of estrogen, a common hormone that stimulates bone resorption, can impact negatively bone mineral density. Thus, there is a link between diet, menstrual cycle, and bone health (Mallinson et al., 2013; Nattiv et al., 2007; Sundgot-Borgen, 1994).

Body mass is another factor that has an impact on BMD. In fact, Tucker, Fosson, Bailey, and Lecheminant (2013) found in non-smoking, non-pregnant, 35-45 years old females, BMD was most affected by body mass and not age, height, menopausal status, physical activity, calcium or vitamin D intake, or the use of Bone Prescription Drugs (Tucker, Fosson, Bailey, & Lecheminant, 2013). Their findings suggest that females with a higher body mass also have a higher BMD, while females with a low body mass tend to have lower BMD. This is due to lighter weight females not placing enough stress on bones during weight bearing activity compared to heavier females so PA will not stimulate adaptations leading to stronger bones. Thus, forces placed on bone have a role in BMD. In fact, Bailey and Brooke-Wavell (2010) observed premenopausal females age 32.6 ± 8.5 years and found that BMD at superolateral hip sites was related to muscle function and maximal impact forces while BMD at inferiomedial hip was mostly related to body mass and hip geometry to FFM (fat free mass) (Bailey & Brooke-Wavell, 2010). It is important to understand BMD in different parts of the body and the difference between sites in the same bone because physical activity may improve BMD at one site while body mass, hip geometry, and FFM impacts a different area.

While physical activity is important to bone health during adolescence, it is relatively unknown what impact physical activity has on BMD later in life if females are active during adolescence and inactive as adults or vice versa (Bailey & Brooke-Wavell, 2010). Specifically, are females who were active as adolescents also active as adults? Is there a time period when activity levels decline that may impact bone health? While much research has been obtained on these subjects in young girls, college level athletes, and post-menopausal females, little has been done on females during their childbearing years. The purpose of this study was to determine the impact of physical activity and nutrition during adolescence on current bone health. More specifically, we examined the following:

a) The relationship of physical activity during adolescence to current BMD;

b) The relationship of current physical activity on BMD;

c) The relationship of calcium intake during adolescence to current BMD;

d) The relationship of current calcium intake on current BMD, in 30-50 year old premenopausal females.

Literature Review

Introduction

Next follows a review of previous literature concerning bone mineral density (BMD) or bone mineral content (BMC) and how it is affected by various factors throughout life. Main factors include nutrition and physical activity (PA), and optimal practices are more important during adolescence compared to early adulthood for a healthy adult skeletal. Healthy bones during adulthood depend both on accumulating a maximal amount of bone mineral during adolescence and early adulthood and maintaining the BMC by a low BMD decrease rate facilitated by staying physically active. Bone growth during adolescence will be discussed before moving on to the relation between bone growth and physical activity. After that follows a section on PA during adolescence and its impact on bone during adulthood. Then, bone density will be discussed in relation to diet and body mass.

Bone Growth

To understand bone health, it is important first to understand bone growth and factors affecting bone development. Bone growth is essential to reach a healthy BMD. To promote bone growth, we first need to know what stimulates bone growth and development and the maintenance of a healthy BMD. After an overview of bone growth in general follows a section on bone growth specifically during adolescence and the importance of this time period.

General bone growth depends on bone formation by which there are two main mechanisms (Kemper, 2000): a local mechanism resulting from mechanical forces of gravity acting on the body and the corresponding muscle contractions and a central hormonal mechanism with estrogen production. The first mechanism uses forces of gravity and muscle contractions, so it is connected to physical activity; but hormones promoting bone development also increase with physical activity, so both mechanisms are relevant to this paper (Kemper, 2000).

The first mechanism of muscle contraction works directly on the bones by applying a force that stresses bone and then affects the activity by osteoclasts and osteoblasts (Kemper, 2000). If the amount of stress is adequate, the activity by osteoblasts is greater than the activity by osteoclasts, resulting in bone mass deposition and increasing bone mass. This force placed on bone is sensed by osteocytes by the flow of interstitial fluid caused by strain. Osteocytes stimulate osteoclasts to remove damaged parts of bone and osteoblasts to repair the bone structures. If osteocytes are over stimulated by either a training stimulus that is too high (intensity) or too high a training volume (duration or repetitions and sets), the activity of osteoclasts is greater than the osteoblasts, so the stress placed on bones may lead to micro fractures, which will not be repaired by mineral deposition. On the other hand, if stimulus is within an appropriate range of intensity and volume, osteoblasts will cause an increase in bone mass. Adequate physical activity and muscle contractions stimulate osteoblasts so bone develops and increases in mass.

Mechanical forces placed on bone promote calcium deposition, but another factor also plays an important role in bone growth. The second mechanism promoting bone growth is hormones that maintain serum calcium levels within a limited range (Kemper, 2000). Almost 99% of calcium in the body is deposited in bones (Kemper, 2000). Estrogens suppress the activity by osteoclasts, the cells that cause bone calcium resorption, and therefore help maintain or increase bone mass. Physical activity results in serum levels of testosterone and estrogens to be elevated, which affect calcium homeostasis by controlling the activity of osteoclasts and osteoblasts (e.g., decreasing bone mass and promoting increases in bone mass respectively). Also, because of the role of calcium, an adequate calcium intake is essential for increasing bone mass. Hormones increasing as a result of being physically active support bone growth by increasing calcium deposition in bones, but for this to function, an adequate calcium intake is essential.

These two mechanisms of increasing BMD by muscle contraction and hormone levels are interrelated (Kemper, 2000). Physical activity causes muscle contractions and increased serum levels of estrogen that reduce the sensitivity of bone to parathyroid hormone and osteoclast activity (Kemper, 2000). Then, the bone absorbs calcium and phosphorus from the blood. This leads to a lowered blood level of calcium and phosphorus, which stimulates parathyroid hormones that inhibits vitamin D production and then stimulate calcium absorption from bone and decreased bone absorption (Kemper, 2000). Because of the relationship between the two mechanisms, bone health seems to mostly be affected by physical activity that has an effect on both mechanical loading and the hormonal mechanism. Activities that provide a stimulus to bone include jumping, skipping, and stair climbing, all of which may have more impact than walking, bicycling, and swimming (Fuchs, Bauer, & Snow, 2001; Gunter et al., 2012). Physical activity promotes bone development by gravitational forces and hormone increases with high impact exercises.

Females usually have a lower BMD than males, so optimal practices are essential to promote bone health in women throughout life to prevent a low BMD that increases risks for fractures. Bone strength and fracture risk are affected by internal structure of bone mineral, bone density, and quality of bone protein (Nattiv et al., 2007). A low BMD, or osteoporosis, is caused either by an increased bone mineral loss as an adult or by not reaching an optimal BMD during childhood or adolescence (Nattiv et al., 2007). In the beginning of puberty, bone mass increases faster in boys than in girls, and boys ultimately reach a higher peak bone mineral density, which give males an advantage when it comes to bone health (Vatanparast et al., 2010). In both sexes, maximal bone mass is reached in the late thirties and then begins to decline. This gradual decrease is greater in females after menopause because of the hormone changes (Kemper, 2000). Because females accumulate less BMC and lose more after menopause, those of an older age are at a higher risk for osteoporosis than males (Kemper, 2000). To prevent osteoporosis, adequate prevention strategies need to be implemented.

There are two main mechanisms that promote bone health by supporting mineral accretion. They are gravitational forces and strain placed on muscle and bone during physical activity and the elevation in hormone levels triggered by exercise (Kemper, 2000). Physical activity is involved in both these mechanisms of supporting bone health so physical activity can be considered important in bone health. Physical activity is important for females since they are more prone to a low BMD and osteoporosis.

Bone Growth and Physical Activity

Physical activity has an impact on BMD as it promotes calcium absorption by bone (Kemper, 2000). Again, the first mechanism of bone growth and the strain placed on bone by physical activity stresses the bones with gravitational forces and muscle contractions (Kemper, 2000). Weight-bearing exercises are needed as they place the necessary forces on the bone and is therefore better than non-weight bearing activities that are low impact (Fuchs et al., 2001; Heidemann et al., 2013; Heinonen et al., 1995; Kemper, 2000). Weight-bearing activities are better for improving bone health in legs and the vertebrae of the lower back (Heidemann et al., 2013; Kannus et al., 1995; Kemper, 2000; Nattiv et al., 2007). Gunter et al., Heidemann et al., and Kemper (Gunter et al., 2012; Heidemann et al., 2013; Kemper, 2000) have shown that bone mass increases proportionally to the intensity of the load. For optimal bone development, physical activity should include high forces that are applied quickly (Gunter et al., 2012; Heidemann et al., 2013; Kemper, 2000). Activities with the most effect on bone health include those applying a ground reaction force greater than 3.5 times the bodyweight (per leg) and peak force should occur within 0.1 seconds (Gunter et al., 2012). For example, Gunter has been shown that 10-15 minutes of jumping, or 100 jumps) from a two-foot height three times per week promotes bone health (Gunter et al., 2012). Since females often don't reach a high BMD compared to males and lose more BMD during and after menopause, it is important for females to participate in weight-bearing activities to promote a healthy BMD and avoid osteoporosis (Kemper, 2000). The best physical activities for bone development are weight-bearing activities including high forces applied at a faster rate, which increases strain on bone, and promote adaptation (Fuchs et al., 2001; Heidemann et al., 2013; Heinonen et al., 1995; Kemper, 2000).

Fuchs and Snow (2002) examined the effect of high impact jump training on increases in hip bone mass from age seven until age nine years. Height, weight, body fat percentage, and sexual maturation (assessed using Tanner stages) were assessed. Jumpers completed the 7-month exercise and two months of detraining in which they were encouraged to continue regular activities but no organized jumping was performed (Fuchs et al., 2001). During premeasures, both groups had similar BMC in both lumbar spine and femoral neck. The training intervention caused the jumpers to have a greater gain in BMC during the first seven months of the study. The greater BMC in jumpers compared to controls observed in the femoral neck remained even after 7 months of detraining, while the greater BMC in jumpers in the lumbar spine did not persist at the end of the detraining period (Fuchs et al., 2001).

More studies support jumping or other high impact activities to promote bone health. Heinonen et al., examined the effect on bone with different types of physical activity to determine if higher peak forces and strain rates have a greater effect on BMD (Heinonen et al., 1995). Female athletes participating in squash (n=18), aerobic dancing (n=27), speed skating (n=14), and healthy sedentary controls (n=25) were recruited. Results showed that squash players had the highest BMD, followed by aerobic dancers and finally speed skaters. Athletes in all these sports had significantly higher BMD compared to sedentary controls. Heinonen concluded that athletes in sports demanding higher strain rates in varying movements (such as squash) have a significantly greater BMD compared to sedentary peers (Heinonen et al., 1995). Also, high peak stresses (as in weight training) have a significant positive effect on BMD. Again, training with the greatest effect on bone includes weight-bearing activity when high forces are applied on bone.

Heidemann et al., completed a two-year longitudinal study with 682 children (49% girls and 51% boys) age range 7.2-12.0 years in Denmark (Heidemann et al., 2013). Control schools kept the original PE program, while the six participating schools modified their physical education for the study. Modifications in the sport schools included implementing additional physical education lessons per week and educating PE teachers on training principles relating to different age groups designed by Team Denmark (Wedderkopp et al., 2012). Individuals in sport schools had PE at least 4.5 hours per week, which was divided into at least three different lessons. Control schools kept their standard amount of PE that was 2 lessons per week resulting in a total of 1.5 hours a week. Activity was not specified but it is likely to have been a mix of different sports at a variety of intensity levels. Participants completed a pubertal self-assessment using Tanner staging (Tanner, 1962) to estimate maturation. Physical activity assessments were performed using the Actigraph GT3X accelerometer in the middle of the 2-year PE intervention to categorize participants into different groups depending on activity level. The accelerometer was used by subjects from awakening until sleep time for 7 consecutive days to estimate their average energy expenditure. Activity intensity levels were defined according to set levels in studies done by Evenson et al. (Evenson, Catellier, Gill, Ondrak, & McMurray, 2008). Intensity levels were defined as sedentary activities METs < 1.5, light physical activity 1.5 < METs <4, moderate physical activity 4< METs<6, and vigorous physical activity METs >6.

Results indicated no significant difference between sexes or bone area between the groups at baseline. With treatment intervention, the high intensity group showed a positive relationship with bone area indicating a positive association between physical activity and bone mineral accretion in childhood, particularly with moderate to high physical activity (Heidemann et al., 2013). Further, the results also suggest bone mineral accruement is affected by time spent in different physical activity intensity levels (Heidemann et al., 2013). Moderate to high intensity have a positive effect on bone health while more time performing low-level and sedentary activity instead of moderate to high-level activity will have less or no effect on bone health in childhood (Heidemann et al., 2013). Time in the different intensity levels was found using the accelerometer. In conclusion, more time spent in a high level of exercise intensity has a greater effect on BMD than the same time spent in lower intensity levels of PA.

Bone growth is stimulated by strain placed on bone and the gravitational forces by physical activity (Kemper, 2000). Activities effective for applying this force include weight-bearing and high impact activities, which apply a high force quickly (Fuchs et al., 2001; Heidemann et al., 2013; Heinonen et al., 1995; Kemper, 2000). Bone benefits increase proportionally to the load or impact of the exercise (Bailey & Brooke-Wavell, 2010; Fuchs & Snow, 2002; Heinonen et al., 1995; Kannus et al., 1995; Kemper, 2000). More time spent in higher intensity activities as compared to lower intensities is an advantage for bone development and strong bones (Kannus et al., 1995; Michalopoulou et al., 2013).

Bone Growth and Adolescence

A greater amount of the total bone mass in adults seems to be achieved during and around puberty, when hormone levels change and bone growth is at a maximum. During puberty it is possible for 15% of adult BMD to be deposited on bone in females (Kemper, 2000). Vatanparast et al., stated that bone growth decreases quickly after menarche, and there is no significant bone mass increase two years after menarche in some bone sites (Vatanparast et al., 2010). Adolescence is an essential period for bone growth where bones adapt easily by the two mechanisms of hormones and mechanical loading caused by PA paired together with the natural hormone changes occurring at that time period (Kemper, 2000).

Michalopoulou et al. (2013) observed 82 premenarcheal girls matched for age (10-13 years old), bone age, and maturity level. They were assigned into three groups by different levels of physical activity according to a 4- day physical activity questionnaire (low, moderate, high levels of physical activity). It was found that regular physical activity increases density and size of cortical bone and density of trabecular bone in premenarcheal girls (10-13 years of age) (Michalopoulou et al., 2013). Also, pericortical thickness, cross-sectional area and bone mineral content increases in both cortical and trabecular bone as the level of exercise increases (Michalopoulou et al., 2013). Physical activity in premenarcheal females increases bone content and density, and this increase is higher as the level of exercise increases (Michalopoulou et al., 2013).

Physical activity during adolescence is advantageous to BMD and greatly support BMD increases as this is a time period when bones are adaptable and easily influenced by PA because bones are developing and hormone levels are naturally elevated which support bone development. Longitudinal studies are less common, so it is difficult to draw conclusions as to what happens with bone at a younger age and how previous and current physical activity affects current BMD in adults. If a high BMD at adolescence will remain in adults whether or not individuals participate in physical activity is unclear.

Physical Activity During Adolescence and Its Impact on Bone During Adulthood

Adolescence is a time period when the body is more adaptable and grows quickly as the individual is approaching adulthood and full growth before growth will decrease and eventually stop (Greer & Krebs, 2006; Kemper, 2000). As stated earlier, bone growth is fastened and bone accumulates 40% of adult bone mass during puberty (Greer & Krebs, 2006), or 15% according to Kemper (2000). PA during this time period increases bone deposition drastically. There are studies examining how mineral deposition during adolescence effect bone mass in adulthood (Bailey et al., 1999; Greer & Krebs, 2006; Kemper, 2000).

A critical period for bone mineral density appears in childhood and adolescence (Bailey et al., 2000; Greer & Krebs, 2006; Nattiv et al., 2007; Vatanparast et al., 2010). In fact, this time period may have long lasting effects on BMD throughout life since it is an age where BMD can be increased and maybe maintained during the life span (Bailey et al., 1999). In females, puberty is the time surrounding menarche and is often defined as three years with menarche occurring in the middle (Kemper, 2000). The importance of the time of puberty on bone mineral content was found in a 6-year longitudinal observation of the relationship between physical activity and bone mineral accretion in growing children. Fifty-three girls and 60 boys during adolescence with varying activity levels were observed to examine the effect of PA on BMD increases. Results indicated children with a higher level of physical activity had significantly greater bone mineral accretion at the time of maximal bone growth (Bailey et al., 1999). Maximal bone growth has been suggested to occur during puberty or in the time right before menarche (Kannus et al., 1995); yet Bailey et al., (1999) suggested the period of peak bone growth was found individually by comparing BMD increases in a subject by several consecutive time periods to find the peak BMD increase velocity. Additionally, authors stated that more than 30% of total adult bone mineral in females is attained during the three years around the pubertal time (Bailey et al., 1999), which is similar to Kemper (Kemper, 2000) stating that 15% of adult bone mass is achieved during adolescence. Physical activity during adolescence promotes a greater BMD increase rate that is related to a greater peak bone mass during adulthood (Bailey et al., 1999).

Baxter-Jones, Kontulainen, Faulkner and Bailey (2008) also suggest that higher levels of physical activity during childhood relates to a greater adult BMD (Baxter-Jones, Kontulainen, Faulkner, & Bailey, 2008). Baxter-Jones et al. examined effects on bone accrual with physical activity during adolescence to young adulthood in children aged 8-15 (Baxter-Jones et al., 2008). They examined whether the most physically active children at the start of the study maintained a higher BMD into their thirties compared to less active peers. One year after participants peak height velocity (PHV), the active females had 7.8% greater BMC at the total body and 14.6% greater BMC at the lumbar spine compared to inactive females (Baxter-Jones et al., 2008). Participants were grouped according to their level of physical activity during adolescence and adjusted for age, maturation, height, weight, adult physical activity, calcium intake and BMD measures one year after PHV. Female participants who were most active as children had 15% greater BMC in the total body scan as young adults, as compared to inactive peers during childhood. Further, this indicates that the effects on BMC of physical activity has a greater effect in youth than in adults (Baxter-Jones et al., 2008). Thus, physical activity in children and adolescents improves life long bone health more effectively than physical activity as adults (Baxter-Jones et al., 2008). Physical activity in childhood and adolescence is better for adult bone health that physical activity as adults (Bailey et al., 1999; Baxter-Jones et al., 2008). Again, PA during adolescence is essential for bone health.

It is more effective to be physically active as an adolescent than an adult since bone is more adaptable during this time period (Bailey et al., 1999; Baxter-Jones et al., 2008).

“Older bone is less responsive to loading than is younger bone” (Bauer & Snow, 2003). Since bone adaptations are slow (3-6 months), physical activity interventions should last 6 months at a minimum; but its preferable if they last 1-2 years (Bauer & Snow, 2003). Kannus et al., (1995) included female tennis and squash players to examine the effect of biological age (starting age relative to menarche) when sport was started to find the difference in BMC between playing and non-playing arm (Kannus et al., 1995). The players’ at 27.7 ± 11.4 years had trained 5 years or more, and they started training more (at least 2 sessions per week) by 16 ± 9 years-old. On average, they trained 4.4 times per week, and each session lasted 80 minutes on average (60-180 minutes). The controls at 27.2 ± 9.2 years of age performed no physical activity affecting only the dominant or non-dominant arm. Kannus et al., had participants complete a questionnaire including information on menstrual history (including menarche), years of active playing, starting age, number of training sessions per week, training intensity, average duration of session, other physical activities, diet, vitamin or mineral supplements (Kannus et al., 1995). Their daily dietary calcium intake was assessed using Micronutrica software (Social Insurance Institution, Helsinki, Finland). Participants were divided into three categories depending on menstrual status: a) normal cycle of 23-35 days, b) irregular menstrual pattern, c) amenorrhea with no menses the last 6 months. Also, players were divided into 6 groups according to starting age in relation to age of menarche. The players had a significantly greater BMC in all the sites in the dominant vs non-dominant side (8.5-16.2%) compared to controls (3.2-4.6%) (Kannus et al., 1995). In the non-dominant arm alone, there was no significant difference between players and controls. Further, the BMC showed a clearly decreasing trend as the biological age of start of play increased (Kannus et al., 1995). There was a two to four times greater BMC in players starting before or at menarche compared to players starting 15 years or more after menarche as adult females (Kannus et al., 1995). Stress on bone should be applied before or during puberty for the most benefits on calcium accrual and a greater BMC later in adulthood (Kannus et al., 1995). Bone is more adaptive to loading during adolescence so PA and stress should be placed on bone during this time period as the rate of calcium accrual is greater.

Participating in physical activity during childhood is one way to prevent osteoporosis later in life because the benefits achieved during childhood persist after cessation of activity (Gunter et al., 2012). For optimal bone improvements, this stimuli should be applied during pre- and early puberty, since this is when the skeleton has the greatest response and adaptation capabilities (Gunter et al., 2012). Further, bone benefits achieved through physical activity during childhood persist into young adulthood (Gunter et al., 2012). Animal studies indicate that these benefits remain even after skeletal maturation, but this remains to be proven in human beings (Gunter et al., 2012). In early adulthood, males and females active as children had a 8-10% greater BMC at the hip compared to less active peers (Gunter et al., 2012). Bone health during adulthood is improved with physical activity during adolescence.

Physical activity during childhood and adolescence promotes bone health in adulthood (Bailey et al., 1999; Baxter-Jones et al., 2008; Gunter et al., 2012; Kannus et al., 1995). This is a time when bones adapt easily to stimuli provided by exercise and achieving a higher BMD during this time period tends to support a high BMD throughout life independent of later physical activity levels. Before puberty or the three years surrounding puberty seem to be the most advantageous age period to engage in PA (Bailey et al., 1999; Greer & Krebs, 2006; Gunter et al., 2012). Therefore, physical activity and higher impact activities should be promoted during adolescence along with adequate nutrition to support bone development.

Bone and Diet

Diet is another factor affecting bone health and bone formation. Important micronutrients for bone health include calcium and Vitamin D (Koo & Walyat, 2013). Calcium is essential for bone formation, and 99% of the calcium in the body is found in bones (Kemper, 2000). As more calcium is deposited, the BMC increases and promotes bone health (Kemper, 2000; Nattiv et al., 2007). Calcium mainly comes through the diet, so nutrition habits should be adequate (Bailey et al., 1999; Vatanparast et al., 2010). Also, Vitamin D is important for bone health and calcium accretion as it promotes calcium absorption (Koo & Walyat, 2013).

As stated, physical activity is important for bone development. Weight-bearing physical activity has a greater effect on bone mass than non-weight-bearing activity such as swimming, which puts less physical strain on the bones (Greer & Krebs, 2006). It is unclear if varying levels of calcium intake influence to what extent physical activity has on bone or if exercise alone increases bone mass independently (Greer & Krebs, 2006). In other words, do both PA level and calcium intake need to be adequate for an optimal bone growth? Physical activity and nutrition are both important for bone health (Bailey et al., 1999; Nattiv et al., 2007; Vatanparast et al., 2010), but it is unclear if one has greater impact than the other. Greer et al. state that regular weight bearing activity was shown to have a greater influence on BMD in comparison to calcium intake in children (Greer & Krebs, 2006). Vatanparast et al. (Vatanparast et al., 2010) suggests an adequate intake of calcium during the growth period is essential for maximizing peak bone mineral density (Vatanparast et al., 2010). Recent evidence suggest that low calcium may increase the risk for bone fractures (Greer & Krebs, 2006). Also, adequate vitamin D intake is essential for optimal bone growth and peak bone mass (Koo & Walyat, 2013). Again, bones are mostly made up of calcium, so adequate calcium needs to be consumed along with vitamin D to support calcium absorption and calcium deposition in bones.

During adolescence, peak bone mass must be reached to lower the future risk of developing osteoporosis (Vatanparast et al., 2010). Calcium is important in bone health since it is deposited in bone and causes increased bone strength as more is accrued and BMD increases. A low intake of calcium may lead to a deficiency which causes a decreased bone growth compared to a normal response when a load is placed on bones which then ultimately causes less bone mass accumulation (Kohrt, Bloomfield, Little, Nelson, & Yingling, 2004). Since peak calcium-accretion is at an age of 12.5 years (Bailey et al., 2000; Greer & Krebs, 2006; Vatanparast et al., 2010), it is essential to consume adequate calcium during this time (Vatanparast et al., 2010). An adequate calcium intake needs to be consumed during adolescence.

In addition to calcium and vitamin D, nutrition should be adequate in overall energy intake. Energy intake and availability needs to be sufficient for bone growth to allow an increasing BMD during the growth period (Greer & Krebs, 2006) and during adulthood to maintain bone mineral (Nattiv et al., 2007). Energy availability is important for bone health because it supports eumenorrhea, a healthy menstrual cycle, and estrogen production which in turn prevents bone calcium resorption and stimulates the production of bone formation promoting hormones (Nattiv et al., 2007). An adequate energy intake is essential to support the menstrual function and hormone levels to promote bone health.

Low energy availability impairs bone development and health by causing primary or secondary amenorrhea. With a disrupted menstrual cycle hormone levels changes that in turn decrease bone formation and calcium resorption. Eventually it causes BMD to be below average because of a decreased or reversed bone mineral accretion (Nattiv et al., 2007). Within five days of the energy availability being reduced 33% from 45 kcal/kg FFM/day to less than 30 kcal/kg FFM/day (which is the energy expenditure in resting metabolism in young healthy adults) in young females lutenizing hormone pulsatility is disrupted (Nattiv et al., 2007). A sufficient energy availability is essential to support bone mineral accretion and hormones associated with this process (Nattiv et al., 2007).

If energy intake is not sufficient, estrogen levels are decreased, which in turn impacts BMD by an increased bone resorption (Mallinson et al., 2013; Nattiv et al., 2007). Estrogen deficiency is probably only responsible for a small part of the unhealthy bone changes accompanying amenorrhea in athletes, but is the primary cause of osteoporosis in postmenopausal women since it increases bone resorption (Nattiv et al., 2007). Estrogen deficiency in young females with amenorrhea often has chronic under-nutrition which decreases the rate of bone formation (Nattiv et al., 2007). Bone resorption increased when energy availability was reduced to suppress estradiol (a main subtype of estrogen), and bone formation was then reduced in a dose-response relationship as energy availability decreased (Nattiv et al., 2007). For each missed menstrual cycle, BMD decreases and this might not be completely reversible (Nattiv et al., 2007). Further, physically active women with menstrual irregularities and/or low BMD more commonly experience stress fractures and amenorrheic athletes are at a two to four times greater risk for stress fractures than eumenorrheic athletes (Nattiv et al., 2007). To conclude, an adequate energy intake is essential with a nutritious diet to maintain menstrual function and support a healthy BMD.

Diet is important for bone health; and as the bone grows, particularly during adolescence, nutrition needs to be adequate to support strong bones. Vitamin D is essential to support calcium absorption and bone accruement. Calcium is important to allow an adequate supply for bone mineralization and other body function. Also an energy availability supporting all body function is essential for allowing and maintaining bone development since bone development is not a priority and only provided for when life-supporting functions are met. Adequate nutrition is important throughout life in order to maintain bone health after age 30 when it should have been fully developed.

Bone and Body Mass

Body mass is related to energy availability and is another factor that has an impact on BMD. Tucker et al. (2013), performed a cross-sectional study to examine several factors' effects on the relationship between body mass and hip BMD. The 262 participants were non-smoking, non pregnant, 35-45 years old females with approximately 90% being white, 80% married, and 32% had some college education. Hip BMD was measured using a dual X-ray absorptiometry (Hologic QDR 4500W bone densitometer, Hologic Inc., Bedford, Massachusetts), and body mass was found on an electronic scale (Tanita) to the closest .05kg. Physical activity was measured for seven consecutive days with an accelerometer. Participants' calcium and vitamin D intake was found by having them complete a Block food frequency questionnaire (NutritionQuest, Berkeley, California). They also completed a questionnaire on menopause status and usage of prescribed drugs to enhance BMD in the last 10 years. They found that BMD was mostly affected by body mass and suggest that females with a higher body mass also have a higher BMD while females with a low body mass tend to have lower BMD. It was concluded that it is problematic for females with low body mass and BMD since lifestyle choices may not help. Further, gaining weight is likely to increase BMD, but it not a recommended method for improving bone health (Tucker et al., 2013). It is stated that more research is needed to determine whether or not modifications in diet have the potential to affect BMD (Tucker et al., 2013). Body mass has a large effect on BMD as it affects the amount of mechanical loading on bones with weight bearing activity.

It was previously known that BMD is positively affected by muscle mass and strength (Bailey & Brooke-Wavell, 2010). Further, Bailey et al. (Bailey & Brooke-Wavell, 2010) observed healthy premenopausal females and found a relation between BMD and muscle function. Muscle function refers to more than just muscle strength. Bone and muscle interactions are affected by the neuromuscular system, hormonal factors, stimuli (either extrinsic or intrinsic), or genes regulating bone and muscle size (Bailey & Brooke-Wavell, 2010). Muscle function was measured as maximal impact forces independently of body mass by looking at maximum ground reaction force after a vertical countermovement jumps. Eighty-eight sedentary premenopausal females age 32.6 ± 8.5 years were measured for fat free mass (FFM), isometric knee extension strength, and peak landing ground reaction force. BMD was measured at the proximal femur and sub regions, section modulus (indicator of bone bending strength), and cross-sectional area (indicator of bone strength during axial stress) were measured using dual X-ray absorptiometry (GE Lunar, Madison, WI, USA). It was a cross-sectional study investigating associations between BMD, bone geometry, muscle function, body size, and body composition. A questionnaire was used to find information about previous and current physical activity. For previous activity, participants rated their level of physical activity; and for current activity participants were asked for duration and frequency. This study was done since it has been difficult to examine the effect muscle has on BMD compared to the effect of mechanical loading on bones by physical activity on BMD. Further, individuals not physically active but with larger and stronger muscles are able to produce more powerful movements (e.g. fast stair ascent/descent) and therefore also higher impact forces for brief moments throughout the day that may affect bone health (Bailey & Brooke-Wavell, 2010). It is known that body mass affects BMD; but the effect of PA, separated from muscle strength, such as mechanical loading, considered separately and independently of body mass, is less clear. It is problematic for females with a low body mass to support bone health because weight bearing activities such as running may not put enough strain on bones to promote bone strength.

In conclusion, PA and diet are important to promote bone health and decrease the risk for low bone mineral density and its related diseases, such as osteoporosis, and fractures. PA should include weightbearing activities that place high loads on bone, and the activities should be accompanied by a well-balanced diet including adequate amounts of calcium. The time period especially important for bone health is adolescence when the calcium accretion rate is at its highest, leading to growth, and bone is more responsive to stimulus and adapts more easily. Developing strong bones during adolescence tends to promote bone health in adulthood as bone growth during this time period tends to be maintained.

Chapter 2: Experiment

Methods

Participants in this study included premenopausal females aged 30-51 years. Peri- and post-menopausal females were excluded due to the relationship of estrogen to BMD. The participants were recruited through word of mouth and e-mail announcement for faculty/staff members of Appalachian State University.

In order to participate in this study, women had to be between 30-50 years old and premenopausal, with no major disease or condition that could have a significant effect on the present investigation (e.g., cardiovascular disease, cancer, muscle condition, or anything limiting PA or PA adaptations). Once they were informed about the study, they contacted the Principal Investigator via e-mail or phone to determine their eligibility for the study. If they fit the inclusion criteria, a time to meet and complete the consent forms as well as initial testing was scheduled. The university's Internal Review Board approved all procedures.

Procedures

Participants met with the Principal Investigator in the Neuromuscular Lab twice. The first session consisted of signing the Informed Consent (Appendix B) and undergoing the Dual Energy X-Ray Absorptiometry (DEXA) scan (Hologic, Bedford, MA). After the scan they also completed the first survey (Appendix C) on demographics, menstrual history, and physical activity habits. Upon completion, they were provided a physical activity history questionnaire (Appendix D) and a diet recall survey (Appendix E). A second meeting date was scheduled. During the second visit, participant's bone health was explained, and the diaries were returned and evaluated.

The DEXA was used to determine total bone mineral density. The same investigator conducted all testing and analyzed all scans. The DEXA was calibrated prior to each participant, and the average reliability reported during the testing procedures was ±1.5% of the mean at 1.001 g/cm^2 with SD 0.003 from the lumbar spine phantom. The coefficient variability was 0.311%.

The survey completed during the initial visit included basic demographics as well as addressed information regarding the menstrual cycle, fracture history, and physical activity participation. Specifically, questions addressed demographics (e.g., race, marital status, and highest grade of school completed), menstrual cycle history (e.g., age of menarche, menstrual cycle regularity, use of birth control pills, previous pregnancies), fracture history (e.g., stress fractures or broken bones), previous sports participation (e.g., sports played in high school and college, level of play), and current physical activity participation including intensity and frequency (see Appendix C). Current PA questions asked about moderate and vigorous intensity PA (Godin, Jobin, & Bouillon, 1986). Moderate PA was defined as activities causing small increases in breathing or heart rate (e.g., walking, volleyball) performed for at least 10 minutes continuously. Vigorous PA was defined as activities causing large increases in breathing or heart rate (e.g., running or swimming) performed for at least 10 minutes continuously.

Participants were sent home with three additional items. The first item was a survey that asked about previous physical activity participation during ages 14-21 years, 22-34 years, and 35-50 years (Appendix D) (Kriska et al., 1988). Time spent being active was calculated from this report as hours per year during the different age categories. The second item was a nutrition recall asking about dietary habits during high school to determine the frequency of consumption of foods with high calcium content (Appendix E) (Nurses Health Study, 2004). This was quantified by summing all seven food items in the survey about dairy with each answer analyzed using a point system (points were awarded for each food item in a food group and summed for a total score for each food group). There were nine different choices of consumption frequency for each food item. Possible answers ranged from “never, less than 1 per month” to “more than 6 per day.” A similar quantification method was used for the grain group and vegetable and bean group (taking into account that grains had 11 different food items and therefore increasing the maximum total of points to 88). The vegetable group had seven food items leading to a maximum total of 56 points. Grains included cold breakfast cereal, hot breakfast cereal, white bread, dark bread, English muffins or bagels, muffins, corn bread or corn toasties, biscuits rolls, rice, tortillas, and pancakes and waffles. The vegetable and bean group included string beans, beans or lentils, broccoli or brussel sprouts, peas or lima beans, raw spinach, cooked spinach, and kale or chard greens. The third item was a diet diary (Appendix F) where participants were asked to fill out a 3-day (pen and paper) nutrition diary using two weekdays and one weekend day.

All nutritional analysis was performed using a nutrition analysis program, The Food Processor SQL. Based on the self-reported three day food diary from each participant, foods were entered into the program as detailed as possible (e.g., cooking method, brand name, restaurant chain and entrée when applicable) with specified quantity. The program analyzed the information and provided total calcium consumed compared to recommended levels.

Important outcome variables included PA time during adolescence as average hours per year ages 14-21 years, current PA time in minutes for moderate and vigorous intensity

PA, previous calcium intake as points based on possible total, and current calcium intake as a percentage of recommended value.

Statistical analysis

Basic descriptives with means and SD were calculated to provide initial group averages, while other demographics were reported as frequencies. Pearson product correlations were performed to determine potential relations between current BMD and previous PA (average/year), current PA (total time moderate PA and total time vigorous PA), previous calcium consumption (total consumption of dairy, whole grains, and vegetables).

Results

Twenty-seven women started and completed the study. Tables 1-4 and figure 1 provide basic descriptives and frequencies for all participants

Overall our sample was relatively healthy as indicated by BMI and percent body fat (Table 1). Additionally, they tended to be well educated and married with children (Figure 1 & Table 2); however, 26% reported family history of osteoporosis, and 19% had previously had a stress fracture (Table 3). With regards to current BMD, most women were average or above average according to the BMD Z-score (Table 4). All participants met PA recommendations for moderate and vigorous PA but fell shy on meeting calcium recommendations for adult females (Table 4). Results for previous PA (PA between ages 14-21 years) were favorable in that many were active as teenagers; however, self-reports of intake of foods rich in calcium were acceptable for dairy but low for whole grains and vegetables (Table 4). Of the 27 participants, there was one participant with a BMD Z-score of 4.0 that was excluded and a second participant with average hours PA/year during ages 14-21 years that was also excluded due to incorrect reporting.

Nonetheless, Pearson product moment correlations indicated a weak correlation between BMD and previous PA (r=0.37) (Table 5). Current PA, weekly moderate and vigorous intensity separately, were also related with current BMD (r = 0.23; r = 0.23, respectively) (Table 5). Previous dairy and current BMD had a correlation r-value of 0.28 and current calcium and BMD had a correlation of 0.17 (Table 5).

Descriptives (N=27 if not otherwise noted)			
	Mean ± SD	Minimum	Maximum
Age (years)	41 ± 5.88	31	51
Body Weight (kg)	65.90 ± 14.74	46.68	105.54
Height (m)	1.62 ± 0.08	1.52	1.79
BMI	24.56 ± 4.85	19.45	37.78
Age of Menarche (years)	13 ± 2	10.5	16

Table 1: Physical characteristics of pre-menopausal females aged 30-50 years.

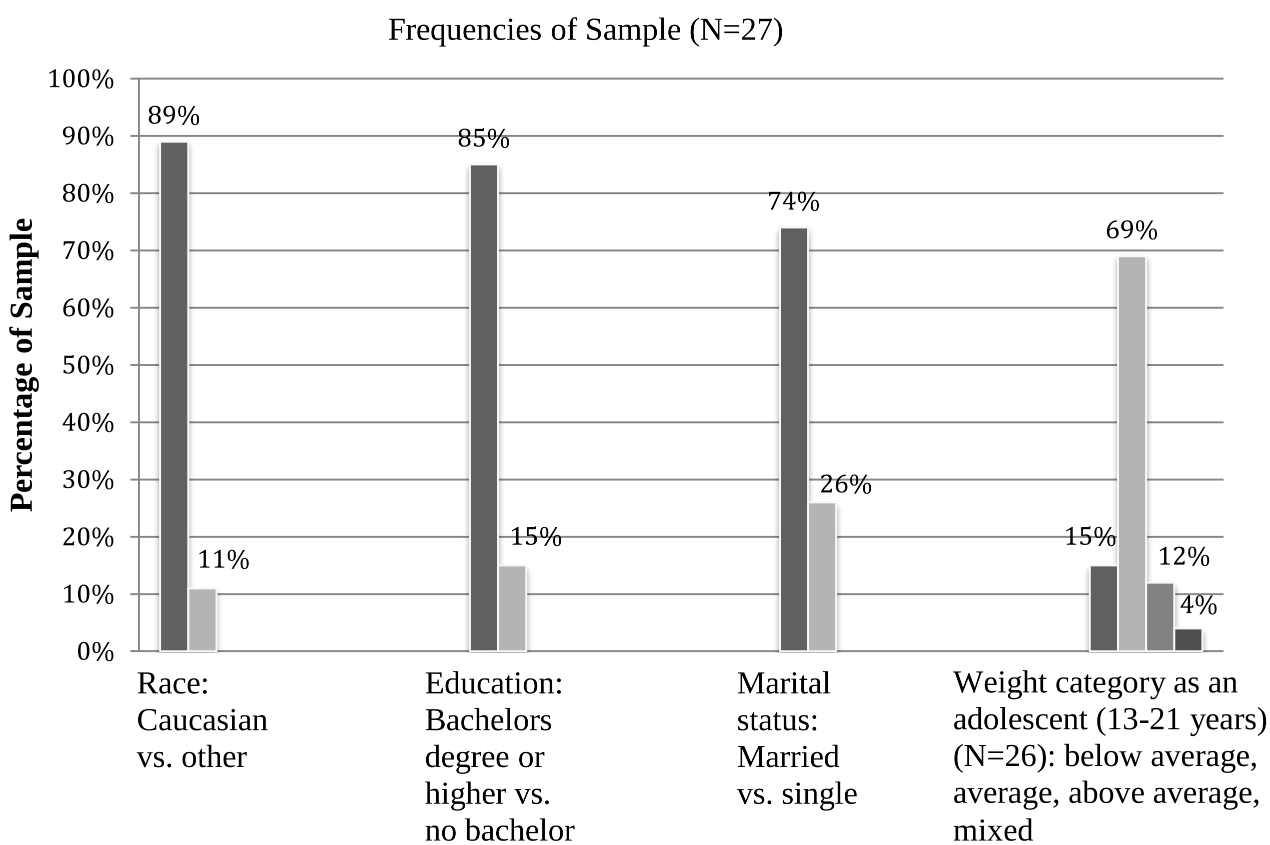

Figure 1: Frequencies regarding background information on premenopausal females aged 30-50 years.

Frequencies (N=27)							
Taken Birth control	74% yes		26 % no				
Been pregnant	67% yes		33% no				
Age of Menarche (years)	4% 10	30% 11	7% 12	37% 13	4% 14	11% 15	7% 16
Age reaching peak height (years)	15% < 14	11% 14	19% 15	26% 16	7% 17	7% 18 +	15% don't know
Maturation Category	33% early ≤11 years old	44% average 12-13 years old	22% late ≥ 14 years old				
Menstrual cycles per year	63% with 12	7% with 11	4% with 10	11% with 5-9	11% with 0-4		

Table 2: Frequencies regarding menstrual cycle including birth control and pregnancies of pre-menopausal females aged 30-50 years.

Frequencies (N=27)				
Osteoporosis in family	26% yes		74% no	
Previous Stress Fracture	19% yes		81% no	
Cups of caffeine (coffee)/day	22% at 0	26% at 1	37% at 2	15% at 3

Table 3: Variables related to overall bone health in pre-menopausal females aged 30-50 years.

Descriptives (N=27 if not otherwise noted)			
	Mean ± SD	Minimum	Maximum
Fat % (N=26)	29.64 ± 6.58	16.5	41.2
Fat Mass (kg)	19.83 ± 8.84	8.48	42.56
Lean Body Mass (kg)	42.96 ± 7.35	32.09	60.58
BMC (g/cm^2)	2.15 ± 0.37	1.35	3.41
BMD Z-score	0.45 ± 1.26	-2.6	4.0
Past dairy intake points (max 56) N=26	11 ± 4	4	18
Past grain intake points (max 88) N=24	17 ± 5	8	26
Past vegetable intake points (max 56) N=27	10 ± 4	3	14
PA hours/year ages 14-21 years (N=26)	249 ± 216	0	776
Current calcium intake (% of recommendation) N=25	79 ± 31	32	149
Current moderate intensity PA (min/day)	40 ± 18	0	60
Current vigorous intensity PA (min/day)	39 ± 24	0	90

Table 4: Current BMD, past PA and diet, and previous PA and diet in pre-menopausal females aged 30-50 years.

Variable	r	r^2	P
PA hours/year ages 14-21 (N=27)	0.37	0.14	0.07
Past diet-Dairy	0.28	0.08	0.18
Past diet-Grains	-0.171	0.03	0.44
Past diet-Veggies	-0.004	0.00	0.98
Current time in moderate intense PA (min/ typical day)	0.23	0.05	0.26
Current time in vigorous intense PA (min/typical day)	0.23	0.05	0.27
Current calcium % intake of recommended value	0.17	0.03	0.42

Table 5: Results of Pearson correlation coefficients for BMD (z scores) and previous PA and diet and current PA and diet in premenopausal females aged 30-50 years

We identified three females with a BMD Z-score of -1.0. Characteristics related to the overall group and these three participants are included in Table 6. Observations concerning factors related to BMD indicate all of these participants had lower levels of PA during adolescence and current PA, as well as lower current moderate and vigorous PA, somewhat lower consumption of calcium rich foods as adolescents and two participants had a current calcium intake below the sample average while one participant had a higher value. Additional factors associated with negative bone health in these women include reported family history of osteoporosis as well as increased coffee consumption.

Variable	Group X ± SD	Participant 1	Participant 2	Participant 3
Pregnancies	67% Prevalence	Yes	Yes	Yes
Birth control	74% Prevalence	Yes	Yes	Yes
Length of breastfeeding (months)	13 ± 12	36	36	34
Menstrual function	70% Eumennorea	Yes	Yes	Yes
Previous stress fractures	19% Yes	No	No	No
Family history of osteoporosis	26% Prevalence	Yes	Yes	No
Daily coffee consumption (cups)	1.44 ± 1.01	2	2	2
PA time ages 14-21 years (average hours/year)	249 ± 216	186	171	24
Current min of moderate PA per day	40 ± 18	20	30	30
Current min of vigorous PA per day	39 ± 24	25	30	0
Past dairy intake points (max 56) N=25	11 ± 4	10	5	10
Past grain intake points (max 88) N=23	17 ± 5	18	20	20
Past vegetable intake points (max 56) N=25	9 ± 3	8	9	6
Current calcium intake (% of recommended)	79 ± 31	60	59	98
Body Weight (kg)	65 ± 15	62	61	47

Table 6: Comparison of frequencies and means ± SD with the three low BMD participants.

Chapter 3: Discussion

Discussion

Overall, none of the correlations were significant and all correlations were weak or non-existent. The Pearson-product indicated a weak association ($r=0.37$, $r^2=0.14$) between PA during adolescence with current BMD in 30-50 years old premenopausal females possibly suggesting the importance of PA during adolescence for adult BMD. While our results found only a weak and insignificant correlation, others have found much larger correlations between adult BMD and PA during adolescence. Specifically, previous studies suggest PA during adolescence has a greater effect on BMD than PA during adulthood and often show positive relationships between PA during adolescence and adult BMD (Bailey et al., 1999; Bauer & Snow, 2003; Gunter et al., 2012; Kannus et al., 1995). Not only is physical activity important but so is the intensity of PA. In fact, activity during adolescence has been found to have an effect on adult BMD with BMD increasing as exercise intensity and load is greater (Kannus et al., 1995; Michalopoulou et al., 2013). Unfortunately we were not able to categorize previous PA into different categories depending on intensity. Nonetheless, one reason for the low correlation between PA during adolescence and current BMD in our participants is related to the difficulty for participants to recall time spent doing PA when during their teenage years and the low variability between participants.

Dairy intake during adolescence showed a very weak association, if any, ($r=0.28$, $r^2=0.08$) with current BMD in our participants. Peak calcium accretion occurs during adolescence in the time surrounding menarche, which, on average, is age 12.5 years in females (Greer & Krebs, 2006). An adequate calcium intake during adolescence is essential to allow calcium accretion for BMD increases (Vatanparast et al., 2010). Further, a BMD-increase during adolescence is maintained into adulthood and has a positive effect of adult BMD (Bailey et al., 1999). A review of studies support the statement that calcium intake during adolescence has a positive correlation with BMD (Uusi-Rasi, Karkkainen, & Lamberg-Allardt, 2013). The above average BMD found in relation to a greater calcium intake during adolescence allowing BMD increases support the statement that dairy or calcium consumption during adolescence positively affects adult BMD (Nattiv et al., 2007; Vatanparast et al., 2010).

We also found the importance of current PA in 30-50 year old females by the weak association with BMD. In fact, current moderate intensity PA and vigorous intensity PA showed the same correlation ($r=0.23$, $r^2=0.05$) with current BMD. Previous studies have shown that intensity of PA has a positive effect on BMD in adolescent and premenopausal females (Bailey & Brooke-Wavell, 2010; Kannus et al., 1995; Michalopoulou et al., 2013). Bailey and Brooke-Wavell (2010) found that an increased ground reaction force promoted a greater BMD in premenopausal females. Heinonen et al., (1995) stated that high peak forces lead to greater BMD scores in young adults. Specifically, BMD is proportional to the load applied to bone (Bailey & Brooke-Wavell, 2010; Fuchs & Snow, 2002; Heinonen et al., 1995; Kannus et al., 1995; Kemper, 2000). However, our results indicate that current intensity had little impact on BMD, instead any activity positively impacted bone. Even though it does not coincide with evidence found in other studies, this indicates the importance of being active and having a high level of PA and that an increased time spent on exercise promotes greater BMD improvements.

An adequate calcium intake in the diet is important for bone health. Consuming foods rich in calcium provide building blocks for bone that allow growth and support other body functions that rely on calcium; therefore, when blood calcium levels are low, it is taken from bones where it is stored. In our participants, current calcium intake was not related to current BMD ($r = 0.17$, $r^2 = 0.03$). Previous findings state that an adequate calcium intake is essential throughout life in order to support the maintenance of BMD (Nattiv et al., 2007). If not enough calcium is consumed it is being pulled away from bone to support other body functions requiring calcium (Nattiv et al., 2007). Most participants in our study had below recommended values for calcium as most females were only obtaining around 75% of the daily calcium requirements. Nonetheless, Uusi-Raso et al., (2013) observed calcium intake in premenopausal females does not show a consistent positive correlation with BMD as it does in other time periods of life, which may explain our low correlation.

While most of the participants had average and above average BMD, three females had low BMD levels (e.g, Z-score <-1.0). Upon further investigation, it was determined that these females also had lower levels of previous and current PA compared to the sample mean. The daily coffee consumption was somewhat higher in the three low BMD participants, and two of the three had a history of osteoporosis in the family and their current body weight was lower than the sample mean. Their lower body weight is a factor influencing their low BMD as low body weight decreases the forces placed on bone with weightbearing activities, which is an important component when promoting healthy bones (Bailey & Brooke-Wavell, 2010). This finding is supported by Tucker et al. who has found a positive correlation between body weight and BMD (Tucker et al., 2013).

Variables that could possibly cause a low BMD, but were not thoroughly examined, include coffee consumption, energy availability, family history of osteoporosis, number of pregnancies, length of breastfeeding, PA levels, specifically around menarche rather than adolescence in general, age of reaching peak height, PA intensity during adolescence, and weight category as an adolescent.

Limitations in this study include the homogeneous sample, as all our participants were relatively well educated and had healthy weights and body composition. Additionally, BMD Z-scores showed a low variability with three participants representing the low BMD group, making it challenging to find variables involved in causing a low BMD. Further, validity and reliability of the questionnaires may have been a weakness because of the difficulty of recalling accurate numbers even though the questionnaires have been used previously. Similar to other recalls, asking participants to consider their previous PA or diet may lead to an underestimation, ultimately impacting our results. On the other hand, the questionnaire asking about current vigorous and moderate intensity might make participants more susceptible to overestimate their numbers. This is because many people think they are more active than they actually are and when they think of PA activity overall and don't try to separate it into different activities less time is probably spent on calculating the actual time.

In conclusion, trends observed support the importance of PA and calcium intake, especially during adolescence, yet the correlations were not as high as has been reported by previous studies. Future research should further examine the effect of PA on adult BMD in different age categories before menopause with a greater number of participants in a more varied sample. Specifically, effort should be directed towards low BMD participants to examine what is happening within this group exclusively.

References

Bailey, D., & Brooke-Wavell, K. (2010). Association of body composition and muscle function with hip geometry and BMD in premenopausal women. *Ann Hum Biol, 37*(4), 524-535.

Bailey, D., Martin, A., McKay, H., Whiting, S., & Mirwald, R. (2000). Calcium accretion in girls and boys during puberty: a longitudinal analysis. *J Bone Miner Res, 15*(11), 2245-2250.

Bailey, D., McKay, H., Mirwald, R., Crocker, P., & Faulkner, R. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *J Bone Miner Res, 14*(10), 1672-1679.

Bauer, J., & Snow, C. (2003). What is the prescription for healthy bones? *J Musculoskelet Neuronal Interact, 3*(4), 352-355.

Baxter-Jones, A., Kontulainen, S., Faulkner, R., & Bailey, D. (2008). A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone, 43*(6), 1101-1107.

Evenson, K., Catellier, D., Gill, K., Ondrak, K., & McMurray, R. (2008). Calibration of two objective measures of physical activity for children. *J Sports Sci, 26*(14), 1557-1565.

Fuchs, R., Bauer, J., & Snow, C. (2001). Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. *J Bone Miner Res, 16*(1), 148-156.

Fuchs, R., & Snow, C. (2002). Gains in hip bone mass from high-impact training are maintained: a randomized controlled trial in children. *J Pediatr, 141*(3), 357-362.

Godin, G., Jobin, J., & Bouillon, J. (1986). Assessment of leisure time exercise behavior by self-report: a concurrent validity study. *Canadian journal of public health = Revue canadienne de sante publique, 77*(5), 359-362.

Greer, F., & Krebs, N. (2006). Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics, 117*(2), 578-585. doi: 10.1542/peds.2005-2822

Gunter, K., Almstedt, H., & Janz, K. (2012). Physical activity in childhood may be the key to optimizing lifespan skeletal health. *Exerc Sport Sci Rev, 40*(1), 13-21.

Heidemann, M., Molgaard, C., Husby, S., Schou, A., Klakk, H., Moller, N., . . . Wedderkopp, N. (2013). The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. *BMC Pediatr, 13*(32), 1471-2431.

Heinonen, A., Oja, P., Kannus, P., Sievanen, H., Haapasalo, H., Manttari, A., & Vuori, I. (1995). Bone mineral density in female athletes representing sports with different loading characteristics of the skeleton. *Bone, 17*(3), 197-203.

Kannus, P., Haapasalo, H., Sankelo, M., Sievanen, H., Pasanen, M., Heinonen, A., . . . Vuori, I. (1995). Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. *Ann Intern Med, 123*(1), 27-31.

Kemper, H. (2000). Skeltal development during childhood and adolescence and the effect of physical activity. *Pediatric Exercise Science, 12*, 198-216.

Kohrt, W., Bloomfield, S., Little, K., Nelson, M., & Yingling, V. (2004). American College of Sports Medicine Position Stand: physical activity and bone health. *Med Sci Sports Exerc, 36*(11), 1985-1996.

Koo, W., & Walyat, N. (2013). Vitamin D and skeletal growth and development. *Curr Osteoporos Rep, 11*(3), 188-193.

Kriska, A., Sandler, R., Cauley, J., LaPorte, R., Hom, D., & Pambianco, G. (1988). The assessment of historical physical activity and its relation to adult bone parameters. *Am J Epidemiol, 127*(5), 1053-1063.

Looker, A., Melton, L., 3rd, Harris, T., Borrud, L., & Shepherd, J. (2010). Prevalence and trends in low femur bone density among older US adults: NHANES 2005-2006 compared with NHANES III. *J Bone Miner Res, 25*(1), 64-71.

Loucks, A., & Thuma, J. (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab, 88*(1), 297-311.

Mallinson, R., Williams, N., Hill, B., & De Souza, M. (2013). Body composition and reproductive function exert unique influences on indices of bone health in exercising women. *Bone, 56*(1), 91-100.

Michalopoulou, M., Kambas, A., Leontsini, D., Chatzinikolaou, A., Draganidis, D., Avloniti, A., . . . Fatouros, I. (2013). Physical activity is associated with bone geometry of premenarcheal girls in a dose-dependent manner. *Metabolism, 62*(12), 1811-1818.

Nattiv, A., Loucks, A., Manore, M., Sanborn, C., Sundgot-Borgen, J., & Warren, M. (2007). American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc, 39*(10), 1867-1882.

Sundgot-Borgen, J. (1994). Risk and trigger factors for the development of eating disorders in female elite athletes. *Med Sci Sports Exerc, 26*(4), 414-419.

Tanner, J. (1962). Growth at adolescence. 2nd edition. . *Blackwell Scientific Publications*.

Tucker, L., Fosson, E., Bailey, B., & Lecheminant, J. (2013). Is the Dose Response Relationship Between Body Mass and Hip Bone Mineral Density in Women Influenced by Diet, Physical Activity, or Menopause? *Am J Health Promot, 13*, 13.

Uusi-Rasi, K., Karkkainen, M., & Lamberg-Allardt, C. (2013). Calcium intake in health maintenance - a systematic review. *Food Nutr Res, 16*(57).

Vatanparast, H., Bailey, D., Baxter-Jones, A., & Whiting, S. (2010). Calcium requirements for bone growth in Canadian boys and girls during adolescence. *Br J Nutr, 103*(4), 575-580.

Wedderkopp, N., Jespersen, E., Franz, C., Klakk, H., Heidemann, M., Christiansen, C., . . . Leboeuf-Yde, C. (2012). Study protocol. The Childhood Health, Activity, and Motor Performance School Study Denmark (The CHAMPS-study DK). *BMC Pediatr, 12*, 128. doi: 10.1186/1471-2431-12-128

Appendices

Appendix A

Consent to Participate in Research

Information to Consider About this Research

Are the Components of the Female Athlete Triad present in 30 to 50 Year Old Women
Principal Investigator: Dr. Becki Battista, Sara Tegner
Department: Health, Leisure, and Exercise Science
Contact Information: Sara Tegner, mailto: tegnersm@appstate.edu
Mentor: Becki Battista, battistara@appstate.edu

What is the purpose of this research?
You are invited to participate in a research study about the components of the Female Athlete Triad (e.g., menstrual cycle dysfunction, low bone mineral density, low energy availability). By conducting this study we hope to learn if the components listed above are present at all in in women aged 30 to 50 years old. Our purpose is to further examine if any of these components differ between women that are active and/or women that have had multiple pregnancies. We anticipate publishing the results from this study in a journal in exercise science.

Why am I being invited to take part in this research? Are there reasons I should not take part in this research?
You are being invited to participate because you are a pre-menopausal female between the ages of 30 and 50 years. If you are pregnant or have a chance of being pregnant, you will not be allowed to participate. If you participate in this study, you will be one of about 40 or 60 individuals to do so. There are limited risks to participating in this study and one of those risks includes a DEXA analysis. We would like to conduct a DEXA analysis (basically a giant X–ray) of your skeleton in order to determine the health of your bones.

What will I be asked to do?
The research procedures will be performed in the Holmes Convocation Center on the Appalachian State University campus. You will be required to come to the Exercise Science and Neuromuscular Labs on the ASU campus twice, with no longer than seven days between. The total time you will be asked to volunteer is estimated to be about 2 hours. If you participate in the study you will be asked to do the following:

Visit 1:
- Fill out questionnaires regarding your menstrual history, physical activity history, and diet and fracture history.
- Complete a dual energy x-ray absorptiometry (DEXA) scan to measure bone mineral density (the strength of your bones).
- Take home a diet and physical activity journal to be completed over the next week, examples of how to complete the journal will also be provided.

Visit 2:
- Bring in your completed diet and physical activity journal over the course of 2 weekdays and 1 weekend day and discuss the entries with the Principle Investigator.

- You will be handed sheets at the end of the first visit to fill out prior to visit 2. Instructions will be given to help you fill out the sheets correctly. The sheets will include a three day food and physical activity recall, including 2 weekdays and one weekend day. During visit 2 you will bring the recall sheets and participate in a brief interview about what you recorded in your journals.

What are possible harms or discomforts that I might experience during the research?
To the best of our knowledge, the risk of harm or discomfort from participating in this study will only be slightly more than you would encounter in everyday life. *The risks associated with a DEXA scan include exposure to small amounts of radiation. DEXA scanning utilizes radiation to obtain an image of your body. Everyone receives a small amount of unavoidable radiation from the environment each year. Some of this radiation comes from space and some from naturally-occurring forms of radioactive water and minerals. The DEXA scan technique gives your body the equivalent of about 4 extra days worth of this natural radiation. The radiation dose we have discussed is what you will receive from this study only and does not include any exposure you may have received or will receive from other tests. If you are pregnant or trying to get pregnant, you should not participate in a DEXA scan.*It is possible that you may find some of our questions upsetting or stressful and answers may be very personal, however you may choose to skip any of the questions you cannot or choose to not answer.

Are there any reasons you might take me out of the research?
There may be reasons we will need to remove you from the study, even if you want to continue. If we learn that you are pregnant, it is required we remove you from the study to prevent unnecessary radiation exposure. We may find evidence leading us to categorize you as post-menopausal or in between pre and post menopause requiring us to remove you from the study. It is required you be removed because it will alter the results.

What are possible benefits of this research?
By participating in this research, you may benefit by learning about your body composition and bone mineral density (strength of your bones). It is beneficial to know bone strength before menopause since bone loss is accelerated during menopause which may result in osteoporosis. Knowing pre-menopausal bone density may assist in future discussions with you and your doctor concerning osteoporosis prevention treatments. In addition, you will learn about your caloric intake and expenditure over the 3 day period. This research should help us learn more about whether pre-menopausal females between the ages of 30 and 50 years old have any or all component s of the Female Athlete Triad. This information will help us understand more about the existence of the triad.

What will it cost me to take part in this research?
It will not cost you any money to be part of the research. The DEXA scan will be free as part of your agreement to participate.

How will you keep my private information confidential?
Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about the

combined information and you will not be identified in any published or presented materials. To ensure that your information is kept confidential, identification numbers but not names will be used on all documents. All data entry and analysis will be conducted with statistical programs using identification. Your files will be stored in Dr. Battista's office under lock and key and identifiable information will be deleted after one year. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information or what that information is.

With your permission, photos may be taken during the study and used in research presentations of the research findings. Your identity will not be revealed when the photos are presented. Please indicate whether or not you agree to having your photo taken for use in research presentations (without name identification):

☐ Yes, I grant permission for my photo to be taken and used in scientific presentations

☐ No, I do not grant permission

Whom can I contact if I have a question?
The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Sara Tegner, the Principal (Student) Investigator, at tegnersm@appstate.edu or the faculty advisor Dr. Becki Battista, battistara@appstate.edu. If you have questions about your rights as someone taking part in research, contact the Appalachian Institutional Review Board Administrator at 828-262-2130 (weekdays), through email at irb@appstate.edu or at Appalachian State University, Office of Research and Sponsored Programs, IRB Administrator, Boone, NC 28608.

Do I have to participate?
Your participation in this research is completely voluntary. If you chose not to participate, there will be no consequences. If you decide to participate in the study, you can decide to withdrawal at any time during the study.

This research project has been approved on 11/14/2012 by the Institutional Review Board (IRB) at Appalachian State University. This approval will expire on 9/24/2014 unless the IRB renews the approval of this research.

I have decided I want to take part in this research. What should I do now?
If you have read this form, had the opportunity to ask questions about the research and received satisfactory answers, and want to participate, then sign the consent form and keep a copy for your records.

____

Participant's Name (PRINT) Signature Date

Appendix B

Menstrual Cycle, Fracture History, and Physical Activity Participation Questionnaire

Participant ID:_______________________ Date:

____

Participant age: __________

DEMOGRAPHICS

1. Which of the following best describes your race?

_____ Caucasian or White _____ Asian _____ African American _____ Mexican American
_____ Native American _____ Other, describe: ____________________

2. What is the highest grade or year of school you completed?

_____ High School _____ some College _____ Associates degree_____ Bachelors degree

_____ Graduate degree Other, describe:

____

3. Are you currently,

   _____ married _____ single

4. At what age did you attain your maximum height (when did you stop growing)?

   <14 14 15 16 17 18+ Don’t know

   (Nurses’ Health Study, 2004)

5. As an adolescent (13-21), what was your weight category?

   ______Below average _______ Average _______ Above average

   ______ Mixed-explain

MENSTRUAL CYCLE HISTORY

1. How old were you when you had your first menstrual cycle?

   __________ years

2. How many days has it been since your last menstrual cycle?

   _____ < 1 week _____ 1-2 weeks _____ 2-3 weeks _____ > 4 weeks

3. Approximately how many menstrual cycles did you average last year?
   12_____ 11 _____ 10_____ 5 - 9 _____ 0 – 4____

4. On average, and consider the past 6 months, about how many days does your cycle normally last?
   ≥ 30 days ____ 26-30 days _____ ≤ 25 days_____
   _____ other

5. Recently, have your menstrual cycles changed with changes in your diet?
   _____ Yes _____ No

6. Recently, have your menstrual cycles changed with changes in your weight?
   _____ Yes _____ No

Please describe any other menstrual irregularities that are not mentioned above:

7. Have you previously or currently taken birth control pills or hormones? If so, how long were they used?
   _____ No _____ Yes (length of time used) _______________

   If these were subcutaneous (patches) or oral, please list the brand.

8. Have you had any pregnancies?
   _____ No (Skip to question 14) _____ Yes. If yes, how many full term pregnancies have you had? _____

9. With your last child, about how long did you breast feed?
   _____ months

   a. What is the current age of your children?

10. How old were you when you had your children?

    _____ 1st child _____ 4th child

_____ 2nd child _____ 5th child

_____ 3rd child _____ 6th child

11. When you were pregnant with your first child, were you active?
_____ never _____ once or twice _____ many times _____ as much as possible

12. When you were pregnant with second child, were you active?
_____ never _____ once or twice _____ many times _____ as much as possible

13. When you were pregnant with your third child, were you active?
_____ never _____ once or twice _____ many times _____ as much as possible

FRACTURE HISTORY

14. Have you ever had a stress fracture?
_____ No _____ Yes If yes, when (year) and where (what bone) did the stress fracture occur?

15. Other than a stress fracture, have you ever had any broken bones?
_____ No _____ Yes If yes, when (year) and where (what bone) did the broken bone occur?

16. Is there a history of osteoporosis in your family?
_____ No _____ Yes If, yes, please list the relationship of the members who were diagnosed with osteoporosis (e.g., mother, grandmother).

17. How often do you consume caffeine?
____ never_____ 1 cup/day_____ 2 cups/day_____ 3 cups/day_____ 4 more more cups/day

PHYSICAL ACTIVITY HISTORY

18. Did you play sports in high school?
_____ No (move to question 17) _____ Yes

a. Were the sports played at a varsity (e.g., High school JV/Varsity or a Travel league or were they just for fun or recreational?

b. If so, what sports did you play?

19. Did you play sports in college?
    _____ No (move to question 18) _____ Yes If yes, what **sports** did you play and how many **years** did you play each sport?

    a. Did you compete at a varsity or club level?

Physical activity during Childhood (≤ 13 years old).

20. Did you play sports as a young child (≤13 years old)?
    _____ 0-1 hour/week _____ 2-3 hours/week_____ 4-7 hours/week_____ ≥7 hours/week

21. As a child, were the majority of the sports you played competitive (e.g. for a team) or more recreational (e.g., for fun)?
    _____ competitive _____ recreational

22. Were you considered more active than others your age and sex during childhood?
    _____ Yes _____ No

23. During childhood did you participate in unstructured physical activity (pick up games, lawn games, playing in the woods)?
    _____ Yes _____ No

24. Did you have any long term illnesses as a child, meaning were you not able to be active for a significant period of time (e.g., broken bone)?
    _____ Yes _____ No

Physical activity during Adolescence (14-21 years).

25. Were you considered more active than others your age and sex during adolescence?
    _____ Yes _____ No

26. As a teenager, were the majority of the sports you played competitive (travel or varsity) or more recreational (leisure or fun)?
    _____ competitive _____ recreational

27. Did you have any long term illnesses as a teenager, meaning were you not able to be active for a significant period of time (e.g., broken bone)?
    _____ Yes _____ No

Physical activity during College Years (18-22 years)

28. Were you considered more active than others your age and sex during college?
    _____ Yes _____ No

29. Did you have any long term illnesses while in college, meaning were you not able to be active for a significant period of time (e.g., broken bone, mononucleosis)?
    _____ Yes _____ No

30. In college, did you strength train?
    _____ never _____ once or twice _____ many times _____ as much as possible

Physical activity after College (22-34 years).

31. Were you considered more active than others your age and sex during ages 22-34 years?
    _____ Yes _____ No

32. Did you have any long term illnesses during this age period, meaning were you not able to be active for a significant period of time (e.g., broken bone, mononucleosis)?
    _____ Yes _____ No

Physical activity between the ages 35-50 years old.

33. Were you considered more active than others your age and sex during ages 35-50 years?
    _____ Yes _____ No

34. Did you have any long term illnesses during this age period, meaning were you not able to be active for a significant period of time (e.g., broken bone, mononucleosis)?
    _____ Yes _____ No

Current physical activity.

35. Considering the past 6 months, on average, how many days do you exercise each week?

    0 __ 1 __ 2__ 3__ 4__ 5__ 6__ 7__

36. Do you do any **vigorous intensity** sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like (running or swimming) for at least 10 minutes continuously?
    _____ No _____ Yes

37. In a typical week, on how many days do you do **vigorous intensity** sports, fitness or recreational activities?
    __________ Number of days

38. How much time do you spend doing **vigorous intensity** sports, fitness or recreational activities on a typical day?
    _____ hours _____ minutes

39. Do you do any **moderate intensity** sports, fitness or recreational (leisure) activities that cause small increases in breathing or heart rate like (walking, volleyball) for at least 10 minutes continuously?
    _____ No _____ Yes

40. In a typical week, on how many days do you do **moderate intensity** sports, fitness or recreational activities?
    __________ Number of days

41. How much time do you spend doing **moderate intensity** sports, fitness or recreational activities on a typical day?
    _____ hours _____ minutes

(Questions 35-41 from GPAQ)

Appendix C

Previous Physical Activity Questionnaire

Activity	Participate regularly?		Age period 14-21 (8 years total) If yes, number of			Age period 22-34 (13 years) If yes, number of			Age period 35-50 (16 years) If yes, number of		
	Yes	No	yrs	mo/yr	hrs/mo	yrs	mo/yr	hrs/mo	yrs	mo/yr	hrs/mo
Swimming											
Walking for Exercise											
Running/ Jogging											
Bowling											
Tennis											
Golf											
Gymnastics											
Aerobic Classes (Step, Spin)											
Cycling (road or mountain)											
Skating (Ice/Roller)											
Hiking											
Softball/ Baseball											
Basketball											
Racquetball/ Squash											
Skiing/Cross-country											
Skiing Downhill											
Dance											
Volleyball											

Lacrosse										
Soccer										
Field Hockey										
Rugby										
Yoga/Pilates										
Weight Lifting										
Heavy Outside Work(e.g.mowing)										
Other (specify)										

(Kriska, et al., 1988)

Appendix D

Diet Recall

For each food listed, check the box indicating how often on average you used the amount specified between ages 13 and 18.

DAIRY	Never, or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Chocolate milk (8 oz. glass or carton)									
Milk (8 oz. glass or carton)									
Instant breakfast drink (1 packet)									
Yogurt (1 cup)									
Cottage or ricotta cheese									
Cheese, include grilled sandwich, cheeseburgers etc (1 slice or 1 oz)									
Cream cheese (1 oz)									

Nurses' Health Study, 2004

For each food listed, check the box indicating how often on average you used the amount specified between ages 13 and 18.

Bread/ Cereal/ Grains	Never, or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Cold breakfast cereal (1 bowl)									
Hot breakfast cereal, like oatmeal, grits (1 bowl)									
White bread (include bread for sandwiches, toast, French toast, etc.) (1 slice)									
Dark bread (1 slice)									
English muffins or bagels (1)									
Muffin (1)									
Corn bread, corn toasties (1 square)									
Biscuit, roll (1)									
Rice (1 cup)									
Tortilla (1)									
Pancakes(2) or waffles (1)									

Nurses' Health Study, 2004

For each food listed, check the box indicating how often on average you used the amount specified between ages 13 and 18.

Vegetables	Never, or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
String beans (.5 cups)									
Beans/Lentils (.5 cup)- include beans in chili, burritos, etc									
Broccoli or brussel sprouts (.5 cups)									
Peas or lima beans (.5 cups)									
Spinach, raw (1 cup)									
Spinach, cooked (.5 cups)									
Kale, chard greens (.5 cups)									

Nurses' Health Study, 2004

For each food listed, check the box indicating how often on average you used the amount specified between ages 13 and 18.

Main dishes	Never, or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
Canned tuna fish (3-4 oz) in sandwich, casserole etc									
Dark meat fish, e.g., mackerel, salmon, sardines, (3-5 oz)									

Nurses' Health Study, 2004

Appendix E

Three-Day Diet Diary

ID #: Date:

Day of Week:

Time of Day	Food/Beverage Consumed	Quantity (Ex. 1 cup)	Method of Preparation

Vita

Sara Tegner was born in Karlstad, Sweden, to Gunnar and Christina Tegner. She graduated from Karlberg Gymnasium in Amal, Sweden, in June 2007. The following spring, she entered Karlstad University, Sweden, to study psychology for one semester. The following fall, she entered Ashland University, Ohio, to study Exercise Science. The fall of 2009, she transferred to Radford University, Virginia; and in July 2012, she was awarded the Bachelor of Arts degree. In the fall of 2012, she began to study towards a Master of Science degree at Appalachian State University while working as a teaching assistant and a strength and conditioning graduate assistant. The M.S. was awarded in May 2014.

Ms. Tegner holds a Certified Strength and Conditioning Specialist certification through the National Strength and Conditioning Association and a USA weightlifting level 1 certification through USA Weightlifting. She resides temporarily in Boone, NC.