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VITAMIN D STATUS AND BONE MINERAL DENSITY IN FEMALE COLLEGIATE DANCERS AND CHEERLEADERS

By

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Bachelor of Science - Nutrition Sciences
University of Nevada, Las Vegas
2012

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Exercise Physiology

Department of Kinesiology and Nutrition Sciences
School of Allied Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
December 2015

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ABSTRACT

Vitamin D Status and Bone Mineral Density in Female Collegiate Dancers and Cheerleaders

by

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Introduction: An athlete's bone mineral density reflects their cumulative history of energy availability, physical activity, genetic predisposition for bone health, and menstrual status, as well as nutritional, behavioral, and environmental factors.

Purpose: To determine if bone mineral density (BMD) and nutritional factors in bone health are different in two groups of female athletes who have comparable body size/weight requirements, but who engage in qualitatively different training regimens.

Methods: Participants were female collegiate athletes who were members of the UNLV Dance team (n=10) or Cheer team (n=9), ages 18-22. Participants vitamin D status was assessed by obtaining a finger prick sample of blood (< 1 ml). BMD for full body, spine and dual femur was assessed by dual energy X-ray absorptiometry (DXA). A calcium and vitamin D intake questionnaire was also completed.

Results: There was no significant difference between the groups for total body BMD (1.23 g/cm^2 dance vs 1.22 g/cm^2 cheer, p=0.70), spine BMD (1.39 g/cm^2 dance vs 1.36 g/cm^2 cheer, p=0.72) or dual femur BMD (1.20 g/cm^2 dance vs 1.11 g/cm^2, p=0.23). Age matched z-scores for total body BMD were also not significantly different (1.46±1.23 dance vs 0.83±0.52 cheer, p=0.19). However there was a significant difference between age-matched z-scores of the dance team vs. non-athlete female controls (1.46±1.23 dance vs 0.19±1.22 control, p=0.033).

Serum vitamin D status was found to be insufficient (10-29 ng/mL) in 74% of the athletes (27 ± 4 ng/mL dance and 25 ± 8 ng/mL cheer). Daily calcium intake was 504 ± 723 mg for dance and 531 ± 236 mg for cheer versus the RDA of 1,000mg/day. Daily vitamin D intake was 256 ± 335 IU for dance and 228 ± 145 IU for cheer versus the RDA of 600 IU/day.

Conclusion: BMD was not significantly different between the low impact dance team and high impact cheer team. These results suggest that the type of activity (low impact dance vs. high impact cheerleading) was not as important for BMD as participating in 20+ hours a week of physical activity. Although the low levels of calcium and serum vitamin D are of concern, the amount of physical activity in these athletes could have counteracted the negative effects of these nutrient insufficiencies on their bone health.

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CHAPTER 1

INTRODUCTION

Osteoporosis is a skeletal disorder characterized by low bone mass and compromised bone strength predisposing individuals to an increased risk of fracture. In the United States it is estimated that over one-half of the total adult population is affected by osteoporosis and low bone mass, also known as osteopenia. Currently 10.2 million adults have osteoporosis and 43.4 million have low bone mass.[1] Of those diagnosed with osteoporosis, 80% are women, who have an increased bone loss rate of 3% per year post-menopause.[2] Although there are a variety of treatment options for those diagnosed with osteoporosis it is very difficult for older women to accrue additional bone mass and strength after menopause. Thus, emphasis is on prevention and optimizing peak bone mass during the formative years from adolescents to around 30 years of age.

Regular exercise in children and adults has shown positive outcomes for bone health and the prevention of osteoporosis. Specifically, individuals who regularly engage in weight bearing activity such as running, jumping, and strength training have greater bone mineral density compared to sedentary individuals.[3] Mechanical forces during physical activity come from both ground-reaction forces and muscle-joint forces putting strain on bone tissue, which has osteogenic or bone-building effects.[4] Physical activity can also increase muscular strength and stability, which can help decrease the chances of falling or subsequent fracturing of bone. If a fall does occur, an active individual still has a decreased risk for fracture, as their bones are less brittle and prone to break compared to a weak and frail individual.[5] Therefore, it is highly encouraged that physical activity during adolescence be continued and maintaining throughout an individual's lifespan.

Athletes in particular have shown the effect that years of physical activity and mechanical loading can have on bone. Athletes participating in weight bearing sports typically have 5-15% greater total body BMD when compared to non-athletes,[6] and in some cases, is reported to be as much as 30% greater when compared to controls. [7] It has been estimated that if this increase in BMD is maintained into an athletes latter years, then their risk of bone fracture is reduced anywhere from 50-80%.[8]

It has yet to be determined if any one sport or type of athletic activity is more beneficial or osteogenic than another. When comparing BMD between different sports, research is often conflicting and studies do not always take all aspects of bone health into consideration. In general, research has shown that sports that have a repeated occurrence of high impact movements, such as gymnastics, seem to have a greater effect on BMD when compared to sports with little or no impact such as cycling and swimming.[9-11] However, there is no consistency throughout the literature when comparing BMD in sports since not all researchers examine training history, injury, menstrual history, dietary intake, or sunlight exposure. Without taking these factors into account it is very difficult to determine if differences in BMD between sports is solely caused from differences in sport-specific mechanical forces or other confounding factors.

Research is also limited on BMD for athletes in recreational sports or sports not sanctioned by the National Collegiate Athletic Association (NCAA). Currently, the NCAA does not recognize dancing or cheerleading as a sport,[12] although research has demonstrated that both activities require similar physical demands as other collegiate sports based on the results of various fitness tests.[13, 14] Both activities have similar body size/weight requirements, but qualitatively different training programs. Participants in both cheer and dance have also shown a high prevalence of disordered eating and pressure to be thin when compared to other sports,[15] as well as high prevalence of vitamin D insufficiency,[16] which could potentially have detrimental effects on their bone health. Therefore, it is important to evaluate these under researched populations and compare how their differing weight bearing activities and other nutritional factors affect their bone health.

Purpose

The purpose of this study is to determine if BMD and nutritional factors in bone health are different in two groups of female athletes who have comparable body size/weight requirements, but who engage in qualitatively different training regimens. A second purpose is to compare BMD of both teams to a control group of non-athletic females.

Research Hypothesis

It is hypothesized that female collegiate dance team members will exhibit significantly different bone mineral density values for full body, dual femur, and spine BMD (g/cm^2 and z-scores) compared to female collegiate cheer team members. Furthermore, it is hypothesized that these two teams will have significantly different full body BMD (g/cm^2 and z-scores) when compared to non-athletic female controls.

Significance of the Study

An athlete's bone mineral density reflects their cumulative history of energy availability, physical activity, genetic predisposition for bone health, and menstrual status, as well as nutritional, behavioral, and environmental factors. Therefore the significance of studying BMD in female athletes is to identify both current bone health status and how BMD is affected by different types of physical activity. Since these two teams are uniquely similar in all other regards other than training, it may be possible to determine if differences in BMD are primarily due to sport-specific mechanical forces and how BMD of dance and cheer teams compares to that of similarly aged non-athletic females.

CHAPTER 2

LITERATURE REVIEW

Functions and Properties of Bone

Bone has various roles that make it fundamental to our health. It is a storehouse for essential minerals and fat, a site for the formation of red blood cells, and provides protection to vitals organs such as the heart. Bone is also the attachment site for tendons, ligaments and muscles, which allows muscles to exert action from a fixed point and provide support for the entire body.[17] This complex tissue oftentimes has simultaneously different characteristics. In order for bone to provide adequate strength and mobility so bone doesn't break when subjected to internal and external forces, it must be stiff and resist deformation. Concurrently, it must be flexible enough to absorb energy and deform enough to withstand load without cracking. When bone is compressed it will shorten and widen, but when bone is under tension it will lengthen and narrow. This is possible due to the unique composition of bone, consisting of small calcium and phosphorus containing crystals bound by a protein collagen matrix.[18] The calcium-phosphate mineral known as hydroxyapatite, makes up 65% of bone, and provides stiffness and the ability to resist compression. The remaining 35% of bone is composed mainly of collagen fibers, with a small percentage (<10%) being composed of non-collagenous proteins. Collagen provides the bone with its elastic properties, which help it deform enough to resist tension when placed under load.[17] As we age there is an increased risk for fracture since bone mineral crystals and collagen undergo biochemical changes that diminish their capacity to provide the appropriate mechanical strength and elasticity that bones need.[19-21] At any age, however, if bone cannot adapt to these forces, then cracking or complete fracture can occur.

Types of Bone

Another unique property of bone is its relatively low weight in comparison to its strength. In order for bones to provide strength and structural support for our musculoskeletal system and be light enough for efficient locomotion, bones are hollow. The dense, calcified tissue forming the outer shell of long bones and vertebrae is known as cortical bone. It makes up approximately 80% of the total skeletal mass and is capable of bearing great loads. Cortical bone defines the shape of bone and provides sites for attachment of tendons and muscles. The remaining 20% of skeletal mass is made up of the lattice-like trabecular bone that is found inside the cortical shell at the end of long bones and vertebral bodies.[22] In contrast to cortical bone, trabecular bone is light, flexible and porous, sometimes described as honeycomb like in shape, which makes it unable to tolerate peak loads. However, trabecular bone is able to deform when loaded and thus is primarily located at sites such as the spine and at the ends of long bones, where almost constant stress from motion and weight bearing occur. Additionally, trabecular bone provides a large surface area for mineral exchange to take place.

Bone Modeling and Remodeling

Bone remodeling is the continuous process of bone formation and bone resorption that occurs throughout an individual's lifetime. Remodeling in adolescents and adults is influenced by many factors including gender, genetics, nutrition, and physical activity. The mechanical stress that exercise puts on bone is positive for deposition because it increases activity of osteoblasts, or bone producing cells. On the other hand, lack of this mechanical stress seen in bedridden patients or astronauts leads to rapid bone loss and osteoclast activity, which removes bone.[23]

Bone is a dynamic tissue that is able to adapt to environmental stimuli as well as repair when structural damage occurs. The mechanisms of modeling and remodeling work in conjunction to optimize strength of the skeleton during our entire lifetime. Modeling can be thought of as the construction phase of bone growth, as it determines bone shape as new bone is being laid down by osteoblasts during growth. It also determines adaptations to physiological influences and mechanical forces, by gradually widening or changing the axis of bone to accommodate repeated stress.[22] Remodeling on the other hand can be thought of as the reconstruction phase, as osteoclast resorption precedes bone formation and continues throughout the lifespan of bone.[18] Bone strength and mineral homeostasis is maintained by remodeling and thus must be almost a continuous process of removal and synthesis. In fact, 20% of the bone surface, in a healthy adult, is undergoing remodeling at any given time.[24] The primary factors influencing these processes are menstrual status, dietary intake, and weight-bearing physical activity.[25] A decline in bone health can be caused by changes or deficiencies in one or all these factors.

Bone Disease

Osteoporosis is caused by a decrease in bone mass due to age related bone loss and/or the failure to achieve optimal peak bone mass. Therefore osteoporosis is preventable if precautions are taken early enough in life. During childhood and adolescence bone growth occurs, as the amount of bone deposited is greater than that resorbed. This continues until an individual reaches their peak bone mass, where bones have reached their maximum strength and density.[26] After reaching peak bone mass, anywhere from 20-30 years old, individuals start to lose more bone than they form, especially for postmenopausal women. High peak bone density reduces the risk of developing osteoporosis later in life. It has been reported that 60% of the risk of osteoporosis can be explained by the amount of bone mineral density acquired by early adulthood.[27] It has been shown in numerous cross-sectional studies that children and adolescents have greater increases in BMD when participating in regular physical activity[28, 29] and high-impact physical activity[30-32]. It is therefore important for individuals to try and attain the highest peak bone mass possible before its inevitable decline.

Also referred to as a silent disease, osteoporosis is often undiagnosed until after a fracture has occurred. Bone fractures, especially in the elderly, are a very serious health consequence that can lead to ill health, disability, and a decreased quality of life. Approximately one in four individuals who have suffered from a hip fracture become disabled and are unable to regain the ability to walk independently. While one in five require long-term nursing home care.[33] A decline in bone health however starts years before a break ever occurs. Consequently, there is an emphasis on maximizing peak bone mass through education, detection and prevention of low bone mass during the formative years.

Bone Mineral Density in Female Athletes

According to the American College of Sports Medicine Position Stand (2007), non-athletes have low bone mineral density with a Z-score below -2, but an athlete's bone mass is considered low with a Z score < -1 and warrants further investigation, even without the history of fracture.[6] This is due to the fact that healthy athletes typically have a 5-15% higher BMD than non-athletes.[25, 34] The International Society for Clinical Densitometry (ISCD) recommended that the World Health Organization (WHO) criteria for diagnosing osteopenia and osteoporosis not be used on premenopausal women and children. Instead, ISCD recommends that BMD in these populations should be expressed as Z-scores in order to compare individuals to age and sex-matched controls. Premenopausal women therefore are termed to have "low bone density below the expected range for age" with Z-scores below -2.0 and a BMD score below -1.0 in an athlete warrants further investigation. Previous epidemiological studies on low BMD have used WHO T-scores for diagnosis, but future studies are now encouraged to use the best-standardized normative database for premenopausal women, Z-scores.[35]

Previous to the updated ISCD recommendations in 2006 on the use of Z-scores to define low BMD, most studies employed the WHO T-scores for diagnosis for osteopenia and osteoporosis in premenopausal women. A systematic review[6] was done of those studies and found the prevalence of osteopenia (T-score between -1.0 and 2.5) ranging from 22%-50% in female athletes compared to 12% expected in a normal population distribution. The prevalence of osteoporosis (T-score ≤ -2.5) ranging from 0%-13% in female athletes compared to 2.3% in a normal population.

Bone Mineral Density and Low Energy Availability

Under nutrition, whether intentional or not, reduces the rate of bone formation plus the resulting decreases in estrogen leads to an increase of bone resorption. That along with other altered hormone levels ultimately leads to low bone mineral density.[36, 37] In a randomized clinical trial in 2004, subjects participated twice; once being administered a balanced diet of 45 kcal/kgLBM/day and then subjects were administered one of three different restricted energy availability (EA) treatments, which were at 10-20-30 kcal/kgLBM/day.[36] For bone marker analysis this study specifically looked at plasma osteocalcin (OC) and serum type I procollagen carboxy-terminal propeptide (PICP) as markers of bone formation and urinary N-terminal telopeptide (NTX) as a marker of bone resorption. All energy restriction groups saw bone resorption increase and rate of bone formation decrease within 5 days, a condition that, if left to continue, may cause irreversible reductions in BMD. Authors noted that amenorrheic athletes reportedly practice daily diet and exercise providing energy availabilities of ~16 kcal/kgLBM/day, a level between the two most severely restricted energy availability treatments administered in this study. In comparison, regularly menstruating athletes self-administer energy availabilities of ~30 kcal/kgLBM/day, so authors suggested that athletes must maintain their energy availability above 30 kcal/kgLBM/ day to maintain bone health. The authors also noted that low BMDs observed in anorexia nervosa patients and amenorrheic athletes had been attributed to their chronic hypoestrogenism, because the principal role of estrogen in bone turnover is to suppress osteoclast activity and this was supported by their research as estradiol levels were seen to significantly decrease in the most extremely energy restricted group and these changes were observed only after 5 days. This study was innovative in that it was the first study to clearly identify a threshold of 30-kcal/kgLBM/ day that female athletes need to consume in order to maintain reproductive and bone health.

Bone Mineral Density in Dancers and Cheerleaders

In the past decade there has been a multitude of studies looking at dancers of all styles and abilities, with the majority being ballet, modern, professional, and collegiate dancers. Hardly any data exists specifically on collegiate dance team members, so it is unclear how they compare in relation to the following studies on BMD in these different populations of dancers. Bone mineral density in dancers has been reported as higher than controls[38-41], lower than controls[42-45], and no difference between dancers and controls.[46, 47] Substantial differences between research methods, protocols, and control groups make it difficult to determine the actual response that the physical activity and mechanical stresses of dance have on bone density.

For example, *Khan et al*[38] looked at retired Australian professional ballet dancers (51.4± 14.3 years) where self-reported questionnaires determined that hours of ballet training per week during childhood was positively associated with BMD. Control groups were matched for age, height, weight and menopause status but a large limitation to this study was the fact that the authors could not account for twenty-years or more where differences between the groups could be the determinant of bone health and not whether or not they danced during childhood. Two similar studies reporting a positive outcome in BMD from dancing[39, 40] looked at non-elite ballet dancers of similar pubertal age (8-11 years). *Bennell et al* found that BMD was in dancers at the femoral neck and hip compared to age matched non-active controls, but there was no difference at the lumbar spine. In 2006, some of the same authors contributed to a follow up study by *Matthrew et al*, where DEXA scans were done biannually for three years and found that BMD in dancers was significantly greater at all sites compared to age matched controls. Both of these studies emphasize how physical activity during adolescence can help to increase peak bone mass throughout adolescence. It is still important to keep in mind that exercise is only one component of bone health and exercising individuals who are genetically predisposed to bone disease or not maintaining appropriate dietary intake to support bone health, are still at risk for osteoporosis.

Nutrition for Bone Health

Diet plays a significant role of attaining and maintaining bone health. Unfortunately, female athletes may not always be consuming enough calories to match their activity-induced energy expenditure. There is not a biological drive to increase food intake when a calorie deficit is created by exercise, as hunger sensations are mediated by oral and gastrointestinal mechanisms.[48] *Hubert et al*., showed that an acute energy deficit in exercising women failed to generate excitatory signals of hunger compared to women who intentionally skipped a 500kcal meal.[49] Therefore, athletes cannot rely on appetite alone as a reliable indicator of energy requirements.

The pressure for female athletes to have a low body weight in aesthetic sports, such as ballet and gymnastics, has been recognized in research since the 1980s,[50, 51] and more recently in other forms of dance and cheerleading.[13, 15, 52] The National College Athletic Association (NCAA) however does not recognize dancing or cheerleading as a sport,[12] although research has demonstrated that both require similar physical demands needed to compete in any other collegiate sport by comparing results of various fitness tests.[13, 14] Both populations have also shown a high prevalence of disordered eating and clinical eating disorders when compared to other sports,[15] which could potentially have detrimental effects on their bone health.

Female athletes not meeting daily caloric needs, even unintentionally, may see a fast decline in both reproductive and bone health. In postmenopausal women, the largest contributor to the onset of osteoporosis is an estrogen deficiency, as estrogen works to suppress osteoclast activity. Premenopausal women can also see a decline in estrogen when they are unable to consume an adequate amount of calories to meet energy expenditure, resulting in a state of energy deficiency.[53] The body's adaptation to this is to redirect energy away from non-essential processes such as reproduction and estrogen stimulation to support more essential processes such as thermoregulation, immune function and cell maintenance.[54] Under-nutrition resulting in decreased estrogen has been shown to reduce the rate of bone formation.[55] *Ihle and Loucks* showed that within only 5 days, exercising women with an energy availability below 30 kcal/kg lean body mass per day, showed a decrease in blood markers of bone formation and an increase in blood markers of bone resorption.[36] Overtime, this breakdown in bone metabolism can cause irreversible reductions in bone mineral density.[24] Specifically, it can be detrimental for females having a low intake of calcium and phosphorus, necessary for bone mineralization, and vitamin D for calcium absorption and muscle strength.[56] Adequate protein intake of 70-100 grams per day is also recommended for optimal bone health.[57] Collegiate athletes especially need to monitor their diet to ensure they continue to build or maintain bone health as they approach their peak bone mineral density.

Calcium

As the most abundant mineral in the human body, calcium plays a crucial role in most bodily functions. Serum calcium levels are tightly regulated and maintained at 2.5 mmol/L in the ionized form (Ca^{2+}) or bound with protein for transportation. Due to this tight regulation, there is no routine biochemical method to assess an individual's calcium status accurately. Only small amounts of calcium are found in the blood and extracellular fluids, as approximately 99% of calcium is found in bone and teeth in the form of hydroxyapatite. This storage form of calcium can be accessed relatively quickly to maintain calcium homeostasis, if dietary intake of calcium is inadequate.[58]

However, adequate dietary intake of calcium is crucial throughout life in order to maintain bone health. Long-term calcium deficiency leads to loss of bone mass and osteoporosis. Children and adolescents especially need to have an adequate intake of calcium to improve bone mass in order to attain a high peak bone mass, and decease osteoporosis and fractures later in life.[59] Calcium intake in teenagers has been shown as a strong indicator of adult bone mineral density.[60] The recommended dietary allowance for calcium in women 11-25 years old is 1,000 mg per day, but unfortunately as high as 90% of adolescent girls and young women do not achieve these amounts on a daily basis.[61-63] A lack of daily intake along with various other factors that negatively influence the absorption of calcium can lead to the body needing to draw on calcium stores. Overtime, if calcium stores are drawn on more than they are replenished then this leads to demineralization and weakening of the bone matrix.

Vitamin D

Vitamin D is a fat-soluble vitamin found in our diet and synthesized in the skin following exposure to ultraviolet B (UVB) light. Vitamin D is not technically a vitamin but a group of secosteroids that have endocrine and paracrine functions.[64] Vitamin D differs from other vitamins in a number of ways. The most important difference being that dietary intake is not the principal source of circulating vitamin D. In fact, there are limited foods containing vitamin D such as oily fish, egg yolks, and sun-dried mushrooms. Limited levels of vitamin D can be found in fortified food sources including milk, fruit juices, breads, and cereals.[65] Consequently, most humans ingest little natural vitamin D and must rely on sun exposure to ensure adequate blood levels.[66]

Endogenous synthesis upon sunlight exposure of the skin is the predominant source of circulating vitamin D in humans.[67] However, skin synthesis is limited by numerous factors, including pigmentation, race, time of day, season of the year, latitude, age, and percentage of the skin surface area available for exposure.[68] These are important considerations for an athlete as their training environment may affect vitamin D production, specifically indoor training, winter sports, and northern latitudes. For most athletes it is not possible for them to live and train in an environment where they can be exposed to adequate levels sunlight needed to naturally produce 25(OH) D, with one exception being Kenyan runners.[67] In fact, Angeline et al., states that the main cause of vitamin D deficiency in athletes is due to a decrease in sunlight exposure, especially for those participating in indoor-sports such as gymnasts, dancers, and swimmers.[16]

Vitamin D's main function in bone health is in its hormonal form, calcitriol or 1,25-dihydroxyvitamin D, where it elevates plasma calcium and phosphate levels required for mineralization of bone.[69] Vitamin D facilitates the intestinal absorption of calcium by mediating active calcium transport across the intestinal mucosa. By increasing intestinal calcium absorption to maintain adequate serum calcium concentrations, vitamin D is essential for optimal bone health. In a vitamin D deficient state intestinal calcium absorption is only 10-15%, while in a vitamin D sufficient state this percentage increases to 30-35% absorption.[70, 71] Thus, a deficiency in vitamin D results in inadequate mineralization of the skeleton and a decrease in bone mineral density increasing the risk of skeletal disorders and osteoporotic fractures.

The latest recommended dietary allowance (RDA) for vitamin D is 600 IU per day for children, adolescents and adults up to 70 years of age.[72] However this is well below the 800-2200 IU recommended by researchers to optimize overall health and maintain serum 25(OH) D concentrations in the sufficient to optimal range.[73] There is convincing support for the belief that current dietary recommendations are too low and need to be adjusted, as higher vitamin D status is associated with multiple positive health outcomes.[74] Many studies have shown that vitamin D deficiencies are still present in populations with a seemingly adequate sunlight exposure and dietary intake of vitamin D,[75-77] suggesting that RDA's may need to be increased and oral supplementation may be needed to reach sufficient levels.

Measuring Vitamin D Status

It is estimated that 1 billion people, including children, adolescents, and the elderly, are vitamin D deficient or insufficient.[71] Serum concentrations of the intermediate form of vitamin D, 25(OH)D, are used to measure ones' vitamin D status. Although there is no consensus on optimal serum levels of 25(OH)D, the US National Osteoporosis Foundation define vitamin D deficiency as less than 10 ng/mL and insufficiency as 10-29 ng/mL. Serum 25(OH)D levels of 30 ng/mL or greater indicate sufficient levels of vitamin D,[16] while 40-100ng/mL are considered optimal. [73] When serum vitamin D levels fall below 30 ng/mL, parathyroid hormone levels are increased, which activates mobilization of calcium from the bone.[71]

Vitamin D Deficiency in Athletes

Numerous studies have recently shown a high prevalence of vitamin D deficiency and insufficiency in athletes of all age groups worldwide. For example, Lovell[78] recently examined eighteen Australian gymnasts and found that fifteen had inadequate vitamin D levels of below 30 ng/mL and of those deficient, six had levels below 20 ng/mL. Calcium intake was also estimated by individual consultations with a dietitian and a food frequency questionnaire. Of the eighteen gymnasts, thirteen had daily dietary calcium intakes below the daily-recommended intake for their age. The authors did not try to estimate daily vitamin D intake but did note that none of the gymnasts were taking any vitamin D or calcium supplements. All gymnasts were classified as having minimal sun exposure but unfortunately values for daily or weekly sunlight exposure was not estimated. Cases of injuries twelve months prior to data collection was however reviewed and thirteen gymnasts were diagnosed with bony stress injuries or stress reactions and one gymnast suffered a stress fracture. Vitamin D deficiency in Australia had previously thought to occur predominantly in only the elderly population but the results from this study suggest that vitamin D status in athletes needs to be considered when evaluating bone mass, bone stress injuries, and overall health.

A high prevalence of vitamin D deficiency has also been seen in elite athletic populations. A recently published study by Maroon et al[79] assessed vitamin D status on a multiracial sample of professional National Football League (NFL) players. The purpose of the study was to confirm data presented in an abstract by Shindle et at[80] by using similar methods to assess another NFL team. The original research was the first to look at vitamin D in this population and found that 30% of the NFL players had deficient vitamin D levels (<20 ng/mL), while 80% had insufficient vitamin D levels (≤32 ng/mL). Players with significantly lower vitamin D levels also had higher likelihood of muscle injuries compared to uninjured players with sufficient levels. Nonblack players had significantly higher vitamin D levels when compared to black players with a mean vitamin D of 30.1 and 20.4 ng/mL respectively. These results were supported by the follow up study where Maroon et al found 26% of players with deficient levels and 69% with insufficient levels. Also corroborated by the study were the results on how dark skin pigmentation influenced vitamin D as black players had significantly higher rates of deficiency when compared to nonwhite players. Unfortunately sunlight exposure and skin exposure was not estimated for the original research or follow up study, which may have explained how even in an outdoor sport that minimal sun exposure is possible if a majority of the players skin surface is being covered by uniforms.

Vitamin D Status in Dancers and Cheerleaders

A study looking at ninety-eight Israeli athletes from a variety of indoor and outdoor sports including dancers found that 73% of all athletes were vitamin D insufficient (<30 ng/mL).[81] Thirty-three out of thirty-five dancers participating in the study had insufficient vitamin D levels, which was the highest percentage out of the ten athletic populations in the study. As a whole, indoor-type sports (dance, basketball, swimming, tae kwon do, judo, gymnastics and table tennis) had nearly twice the rate of vitamin D insufficiency compared to outdoor sports (tennis, soccer, running, triathlon, and sailing) again emphasizing how sun exposure plays a crucial role in our overall vitamin D status.

A longitudinal study by Halliday et al[82], looked at the prevalence of vitamin D insufficiency in National Collegiate Athletic Association (NCAA) Division 1 athletes in Wyoming throughout an academic year. Males and females were divided into indoor athletes (basketball, wrestling, and swimming) and outdoor athletes (soccer, football, cross country, track and field, cheerleading or dance team). Vitamin D was measured at three time points throughout the year, classified as fall, winter and spring. Results of this study showed significant changes throughout the vitamin D status of athletes throughout the year. In the fall, 9.8% of athletes were vitamin D insufficient compared to 60.6% in the winter and 16% in the sprint. Indoor sports also had significantly lower vitamin D levels compared to outdoor athletes, but unfortunately the authors did not report statistical difference between teams due to small sample size. Also interesting was the classification of dance and cheerleading as an outdoor sport, where time spent outdoors for practice or competition was at the highest in the fall at 4.8 ± 1.8 hours per week. The authors discussed that due to Wyoming's sunny and mild climate that athletes are able to train and perform leisure activities outdoors close to solar noon when vitamin D synthesis is most effective[73] and this could explain why vitamin D insufficiency was lower than expected compared to similar studies in other locations.

CHAPTER 3

METHODOLOGY

Subjects and Design

Forty female collegiate members of the University of Nevada, Las Vegas (UNLV) Rebel Girls dance team or the UNLV cheerleading team, between the ages of 18-25, were recruited for this study. Those excluded were pregnant women, women who thought they were pregnant and individuals with cardiovascular, pulmonary, or metabolic disease. All data for the dance and cheer teams was collected pre-season, immediately following yearly team tryouts. Additionally females who did not participate on either dance or cheer team the previous year were excluded. Full body BMD from female non-athlete controls, who met inclusion criteria, was obtained from previously collected data. Characteristics of the participants are summarized in Table 1. All study participants gave written informed consent and the UNLV Institutional Review Board approved the study protocol (#748192-2).

Table 1: Descriptive Statistics

	Dance (n=10)	**Cheer (n=9)**	**Control (n=10)**
Age	20.5 ± 1.35	19.78 ± 0.44	21.9 ± 1.1*
Height (cm)	163 ± 4.0	162 ± 4.0	164 ± 0.07
Weight (kg)	60.75 ± 7.03	59.11 ± 6.39	62.09 ± 15.21
BMI	22.88 ± 2.32	22.57 ± 2.53	22.9 ± 4.11

Values are mean ± standard deviation; *Significantly different than dance and cheer ($p<0.05$)

Bone Mineral Density and Body Composition

Data collected on subjects included age, race, height, weight, body composition, and bone mineral density. Height (Height Rod use – Health o meter Professional, McCook, IL) and weight (Scale – Tanita BWB-600, Arlington Heights, IL) were taken and BMI values were calculated as the ratio of weight (kg) to height (m) squared (kg/m^2). Total body bone mineral density, dual femur, lumbar spine, and body composition (lean body mass, fat mass, bone mineral content, android fat mass and gynoid fat mass) were determined for the whole body using dual energy x-ray absorptiometry (DEXA) with a Lunar Densitometer DPX-L Radiation (Prodigy, Lumar Corp, Madison, WI). To assess the spine and hips, the patient's feet are placed in a brace to stabilize their legs, while their arms are folded across their chest. For the total body scans subjects were positioned on their backs with arms straight and at the sides with palms touching their sides. BMD was expressed as grams per centimeter squared (gm/cm^2). BMD was expressed as grams per centimeter squared (gm/cm^2). Standardized z-scores based on age and race matched controls were used in the analysis.

Vitamin D

Blood samples were collected for the dance and cheer team via finger lancet extracting 600 µL. Blood and was centrifuged at 3000 RPM for 20 min at 4° C. Duplicate samples of plasma (20 µL) were stored at -80° C until all samples were collected for subsequent analysis. Vitamin D was assayed by a commercial ELISA kit (Eagle Biosciences, INC 20A NW Blvd. Ste 112, Nashua, NH 03063) 25(OH) vitamin D was determined. The intra-assay coefficient of variation (CV) was 4.9% at 10.8 ng/mL, 6.9% at 24.6 ng/mL and 3.2% at 64.1 ng/mL. The inter-assay CV was 7.8% at 16.6 ng/mL, 7.0% at 43.5 ng/mL and 8.6% at 67.8 ng/mL.

Calcium and Vitamin D Food Intake and Sunlight Exposure Questionnaires

Questionnaires were created to determine menstrual status (Appendix II, Question 2), estimate average daily calcium and vitamin D intake (Appendix II, page 2), and average weekly sun exposure (Appendix II, page 2). The calcium and vitamin D content of food was obtained from the USDA and from selected food labels. These values were multiplied by the self-reported daily and weekly intake from athletes and averaged to get an estimated total daily intake for calcium and vitamin D.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows analysis software (PASW Statistics Version 20.0, SPSS Inc., Chicago, IL). One-way ANOVAs were run to explore between-group differences for the means of total body BMD in g/cm^2 as well as total body BMD z-scores. To assess multiple comparisons between the groups, a Tukey's post hoc procedure was run. T-tests were run to explore the differences between the dance and cheer teams for dual femur BMD, spine BMD, serum vitamin D, daily calcium and vitamin D intake.

CHAPTER 4

RESULTS

Data was collected on 29 subjects composed of female collegiate dance team members (n=10), cheer team members (n=9) who met inclusion criteria, and non-athletic controls (n=10) to compare total body BMD and total body BMD Z-scores. Results are provided in Table 2 with mean and standard deviations from all three groups for total body BMD. Data collected from the dance and cheer teams was compared for total body BMD, total body BMD Z-scores, dual femur BMD, dual femur BMD Z-scores, spine BMD, spine Z-scores, serum vitamin D, average daily calcium intake, and average daily vitamin D intake. Results are provided in Table 3 and 4 with mean and standard deviations for a comparison between the two teams.

Bone Mineral Density Compared to Controls

There was no significant difference between total body BMD (g/cm^2) between the dance team, cheer team, and non-athletic controls (p=0.134). However, there was a significant difference between the dance team versus non-athletic female controls for total body BMD Z-scores (1.46±1.23 dance vs 0.19±1.22 control, p=0.033). There was not a significant difference between Z-scores of the dance team and cheer team (p=0.441) or between the cheer team and non-athletic female controls (p=0.441).

Table 2: Total Body BMD Results

	Dance (n=10)	Cheer (n=9)	Control (n=10)
Total Body BMD (g/cm^2)	1.23 ± 0.09	1.22 ± 0.09	1.15 ± 0.11
Total Body z-score	1.46 ± 1.23	0.83 ± 0.52	0.19 ± 1.22*

Values are mean ± standard deviation; * Significantly different than dance ($p < 0.05$)

Figure 1: Total Body BMD Comparison

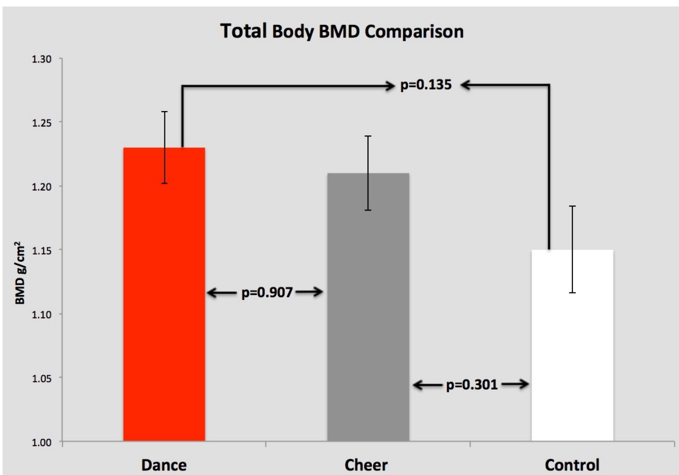
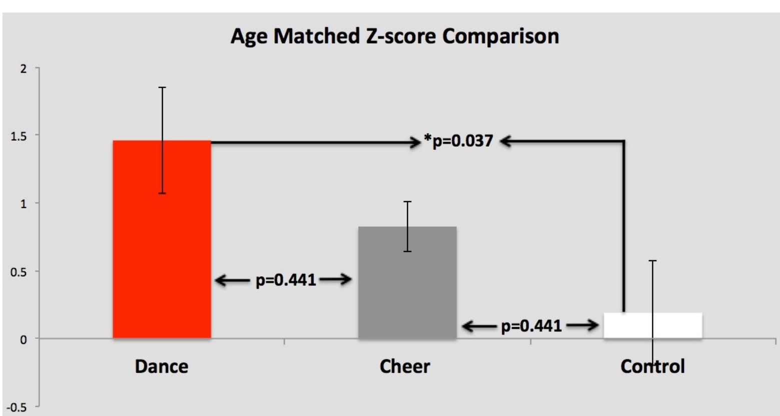


Figure 2: Total Body BMD Z-score Comparison



Comparisons between Dance and Cheer Teams

There was no significant difference between the groups for total body BMD (1.23 g/cm^2 dance vs 1.22 g/cm^2 cheer, p=.70), spine BMD (1.39 g/cm^2 dance vs 1.36 g/cm^2 cheer, p=.72) or dual femur BMD (1.20 g/cm^2 dance vs 1.11 g/cm^2 cheer, p=0.23). Age matched z-scores for total body BMD were also not significantly different (1.46±1.23 dance vs 0.83±0.52 cheer, p=.19).

There was no significant difference between the teams for serum vitamin D (ng/mL). Serum vitamin D status was found to be insufficient (10-29 ng/mL) in 74% of the athletes (27.45 ± 4.26 ng/mL dance vs 24.59 ± 7.61 ng/mL cheer). Only 5 of the 19 athletes had sufficient serum vitamin D above 30 ng/mL.

There were no significant differences between the teams for estimated daily calcium intake (p=0.91) or estimated daily vitamin D intake (p=0.82). Daily calcium intake was 504 ± 723 mg for dance and 531 ± 236 mg for cheer versus the RDA of 1,000mg/day. Only 4 of the 19 athletes had an estimated daily calcium intake near to or above the RDA. Daily vitamin D intake was 256 ± 335 IU for dance and 228 ± 145 IU for cheer versus the RDA of 600 IU/day. Estimated daily vitamin D intake was also low, as only 2 of the 19 athletes consumed above the RDA of 600 IU per day. Estimated sunlight exposure was similar between the groups, and confirmed that both teams can be classified as indoor sports as practices and performances outside are very infrequent.

The questionnaires also revealed that none of the 19 athletes were taking a multivitamin or any calcium supplements. A majority of athletes self reported that they considered themselves to have a normal menstrual cycle, although 3 athletes from the dance team and 2 athletes from the cheer team did not consider themselves to have a normal menstrual cycle.

Table 3: Spine and Dual Femur BMD Results

	Dance (n=10)	Cheer (n=9)
Spine BMD (g/cm^2)	1.39 ± 0.16	1.36 ± 0.16
Spine Z-score	1.48 ± 1.07	0.56 ± 0.62
Dual Femur BMD (g/cm^2)	1.2 ± 0.14	1.11 ± 0.16
Dual Femur Z-score	0.48 ± 0.41	-0.2 ± 1.44

Values are mean ± standard deviation; ($p > 0.05$)

Table 4: Vitamin D Status and Questionnaire Results

	Dance (n=10)	Cheer (n=9)
Serum Vitamin D (ng/mL)	27.45 ± 4.26	24.59 ± 7.61
Daily Calcium Intake (mg)	503.6 ± 723.55	531.33 ± 236.22
Daily Vitamin D Intake (IU)	255.9 ± 335.07	228.22 ± 144.86

Values are mean ± standard deviation; ($p > 0.05$)

CHAPTER 5

DISCUSSIONS AND IMPLICATIONS

Discussion

The purpose of this investigation was to determine if BMD and nutritional factors in bone health are different in two groups of female athletes who have comparable body size/weight requirements, but who engage in qualitatively different training regimens. It was hypothesized that female collegiate dance team members would exhibit significantly different bone mineral density values for full body, dual femur, and spine BMD (g/cm^2 and z-scores) compared to female collegiate cheer team members. Additionally it was hypothesized that these teams would have significantly different full body BMD (g/cm^2 and z-scores) when compared to non-athletic female controls. We found that all bone mineral density measures were similar between dance team and cheer team members. However, when utilizing z-scores only the dance team members were significantly greater than non-athletic controls.

Female athletes engaged in cheer and dance have comparable body size/weight requirements, but engage in qualitatively different training regimens. The cheer team performs movements considered to be high impact, gymnastic based, tumbling movements where both feet are off the ground at the same time and ground force reactions range anywhere from 10-18 times body weight.[83, 84] In contrast, the dance team performs more low impact movements where at least one foot at a time is always on the ground. Small verticals jumps performed by the dance team have ground force reactions of 1.5-3 times body weight.[83] Our results showed that these two groups were almost identical in all other factors that would impact BMD including menstrual status, calcium and vitamin D intake and sunlight exposure. DXA indicated that there were no significant differences between the teams in measures of total body, spine and dual femur BMD (g/cm^2) as well as in the corresponding Z-scores. These results were somewhat surprising as it was expected that the differences between training programs would have a greater effect on BMD. These results suggest that for these athletes differences in mechanical loading were not as important determinants of BMD as the volume of time spent being physically active. Both teams had low calcium and vitamin D intake, and serum vitamin D levels. Although the low levels of calcium and serum vitamin D are of concern, the amount of physical activity in these athletes could have counteracted the negative effects of these nutrient insufficiencies on their bone health.

It is interesting to note that despite insufficient calcium and vitamin D intakes, BMD in the dancers and cheerleaders was greater than that of age matched controls. While the results showed no significant difference between the teams when comparing total body BMD (g/cm^2), both teams had 7% higher BMD than controls. This is consistent with the findings of previous studies indicating that athletes typically have 5-15% higher BMD than non-athletes.[6] Due to the differences in the age-derived standard for Z-scores, there was a significant difference for age-matched Z-scores between the dance team and controls (1.46±1.23 dance vs 0.19±1.22 control, p=0.033).

Although our hypothesis did not predict directional difference between the dance and cheer team it is interesting that our findings indicate that the dance team had the same total body BMD (1.23 ± 0.09 g/cm^2 dance vs 1.22 ± 0.09 g/cm^2 cheer) as the cheer team. However, the dance team had slightly higher Z-scores (1.46 ± 1.23 dance vs 0.83 ± 0.52 cheer) than the cheer team although not significant. Z-scores represent the standard deviation relative to the mean of a reference population taking into account age and ethnicity. Although there is no difference in BMD between cheer and dance, the difference in Z-scores can be explained by differences in the age and ethnicity of the individual members of each team. This may be relevant, since Z-scores are used in the diagnosis of low bone mass in premenopausal women.

Conclusions

Despite differences in training regimens, BMD did not differ between collegiate dancers and cheerleaders in this study. Both groups were insufficient in calcium and vitamin D intake, which could have negatively impacted bone health. However, these findings suggest that the volume of regular physical activity performed by these athletes was sufficient to counteract the deficits in nutrient intake, allowing them to maintain normal BMD status.

APPENDIX I: INFORMED CONSENT

UNLV

INFORMED CONSENT

Department of Kinesiology & Nutrition Sciences

TITLE OF STUDY: Vitamin D Status and Bone Mineral Density in Female Collegiate Dancers and Cheerleaders

INVESTIGATOR(S): Dr. Jack Young and Tara Kenny

For questions or concerns about the study, you may contact Dr. Young at **895-4626.**

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted, contact **the UNLV Office of Research Integrity – Human Subjects at 702-895-2794, toll free at 877-895-2794 or via email at IRB@unlv.edu.**

Purpose of the Study
You are invited to participate in a research study. The purpose of this study is to determine bone mineral density and vitamin D status in female members of intercollegiate athletic teams; the Rebel Girls and UNLV Cheerleaders.

Participants
You are being asked to participate in the study because you fit these criteria: You are currently a member of the Rebel Girls dance team or the UNLV Cheerleaders.

Please understand that even though you may want to participate, there is a chance that you will not be able to. We will use a questionnaire to check to see if you are eligible to participate.

If you are pregnant or think you may be pregnant, you will not be allowed to participate in this study. Similarly, your participation may be restricted if you have had a DEXA scan within the last three months. The reason for this is that bone mineral density is determined using the DEXA scanner, a diagnostic X-ray device.

The UNLV Radiation Safety Office has developed the UNLV Reproductive Health Program to ensure that people occupationally exposed to radiation at UNLV are aware of the risks associated with their exposure. In addition, the principles of radiation protection require that ALL doses (this includes medical examinations) be kept As Low As Reasonable Achievable (ALARA).

This is of particular concern in a study such as this because a developing fetus is especially sensitive to radiation exposure in the first trimester of pregnancy.

The dose that a subject receives from the evaluation of bone mineral density is approximately three (3) millirem. Three millirem is less than 1% of the dose that we receive annually as a result of living in Las Vegas and is 0.6% of the limit for exposure of declared pregnant radiation workers.

The investigators recognize that the risks of participation in this study are very low, but they do not wish to expose a fetus to any unnecessary radiation. For any female, there is a possibility that you are pregnant but do not know that you are. If it is found that you are pregnant after the study, you should know that the potential for damage of the exposed fetus is extremely low. Concern for damage to an exposed fetus is typically expressed at

Page **1** of **2**

Protocol #748192-2, Expiration: 06-04-2016

a dose level of greater than 5000 millirem. The International Commission on Radiological Protection recommends that a one time fetal dose should not exceed 10000 to 20000 millirem.

Procedures

If you volunteer to participate in this study, you will be asked to do the following: Report to the DEXA lab (BHS 335) to undergo a DEXA scan, complete a brief questionnaire on calcium intake, vitamin D intake, a 24 hr dietary recall, and provide a finger stick sample of blood. The finger-stick blood draws of 600 microliters (approximately one-tenth of a tsp each time) will be taken for determination of vitamin D status. The blood sample will be centrifuged; the plasma fraction will be removed and stored frozen until analyzed for vitamin D concentration, after which any remaining sample will be destroyed. Approximately 30 minutes will be required to complete the study.

Benefits of Participation

The direct benefit to you as a participant in this study is knowledge of your bone mineral density. As a result of this study we hope to learn if bone mineral density changes as a result of participation in intercollegiate athletics. You will be provided with a copy of your DEXA scan and are encouraged to consult with the UNLV medical staff or your personal physician if you have any concerns about the results.

Risks of Participation

This study involves minimal risk to you. Other than performing in an experimental setting, this research study does not require you to engage in any activity that is unusual or unfamiliar. You might feel minor pain and/or discomfort during the blood draw, but all equipment will be sterile. There is the possibility of bruising and swelling around the area of the blood draw. The radiation exposure of the DEXA scan is minimal and is approximately the same amount of radiation you receive living in Nevada for less than 3 days.

Cost /Compensation

There will not be any financial cost to you to participate in this study. The study will take approximately 30 minutes of your time. You will not be compensated for your time.

Confidentiality

Only individuals authorized to be in the DEXA room will be present during your scan and blood draw.
All information gathered in this study will be kept as confidential as possible. No reference will be made in written or oral materials that could link you to this study. All records will be stored in a password protected computer in a locked facility at UNLV for 3 years after completion of the study. After 3 years, any documentation with identifiable information (e.g., name) will be destroyed.

Voluntary Participation

Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with UNLV. You are encouraged to ask questions about this study at the beginning or any time during the research study.

Participant Consent:

I have read the above information and agree to participate in this study. I have been able to ask questions about the research study. I am at least 18 years of age. A copy of this form has been given to me.

______________________________ ______________

Signature of Participant Date

____

Participant Name (Please Print)

APPENDIX II: BONE MINERAL DENSITY QUESTIONNAIRE

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

BONE MINERAL DENSITY QUESTIONNAIRE

Department of Kinesiology & Nutrition Sciences

TITLE OF STUDY: Vitamin D Status and Bone Mineral Density in Female Collegiate Dancers and Cheerleaders

INVESTIGATOR(S): Dr. Jack Young, Dr. Laura Kruskall, Dr. James Navalta and Tara Kenny

For questions or concerns about the study, you may contact Dr. Young at **895-4626.**

Purpose of the Study

You are invited to participate in a research study. We want to determine if bone mineral density and vitamin D status is different in two groups of female athletes who have comparable body size/weight requirements, but who engage in qualitatively different training regimens. To better evaluate your participation in this study, please answer the following questions. You may choose to not answer any question.

1. Have you changed your diet significantly in the last 3 months? If yes, what changes did you make?

2. Do you consider yourself to have a normal menstrual cycle?

3. Are you currently taking any medications prescribed by a physician which may interfere with vitamin D metabolism such as oral contraceptives, steroid drugs (prednisone) or anti-seizure drugs (phenobarbital, Dilantin)? If yes, which drug, and how long have you been taking it?

4. Are you currently taking a multivitamin supplement? If yes, how long have you been taking a multivitamin? Which multivitamin are you taking?

5. Have you previously taken or are you currently taking a calcium supplement? If yes, what dose and for how long have you been taking a calcium supplement?

6. Have you ever been diagnosed with low bone mineral density? Are you currently undergoing treatment for osteopenia or osteoporosis?

7. Have you significantly changed your level of physical activity in the past 6 months? If yes, describe the changes you made.

8. Have you had a DEXA scan within the previous three months? If yes, when?

9. For each food listed, indicate how often on average you have used the specified amount.

Calcium & Vit. D Fortified Foods	Serving Size	Servings/Day	Servings/Week
Milk Whole, skim, 1%, 2%, chocolate	8 oz		
Soy Milk/Rice Milk	8 oz		
Vit. D fortified cereal	¾ cup		
Vit. D &/or calcium fortified orange juice	8 oz		
Egg – whole, cooked	1		
Fatty Fish (ie. Salmon)	3 ½ oz		
Yogurt	8 oz		
Cheese	1 oz		
Cottage Cheese	8 oz		
Ice Cream	½ cup		
Other foods – specify			

10. The following questions relate to your regular exposure to the sun. Circle the appropriate response.

How much leisure time is spent outside in the sun?	½-1 hr/day	> 2 hrs/day	1 hr/week	2-4 hr/week	5-6 hr/week
How often do you use sunscreen?	never	sometimes	usually	always	
What SPF sunscreen do you use?	15 spf	30 spf	45 spf	Other (specify)	
What is your race/ethnicity	White	African American	Asian	Hispanic	Other
When you are outside do you typically wear	Long sleeves	Short sleeves	Shorts	Pants	Sports Bra
	Hat				

APPENDIX III: DESCRIPTIVE STATISTICS

Table 5: Descriptive Statistics

	#	Years on Team	Age	Height (cm)	Weight (kg)	BMI
Dance Team	D1	2	19	1.58	50.80	20.38
	D2	1	21	1.55	51.26	21.36
	D3	4	21	1.63	63.68	24.12
	D4	3	21	1.63	70.03	26.52
	D5	4	22	1.67	62.59	22.50
	D6	1	19	1.63	54.43	20.61
	D7	1	22	1.61	66.20	25.54
	D8	1	19	1.68	65.95	23.48
	D9	4	22	1.68	55.79	19.77
	D10	1	19	1.65	66.77	24.52
Mean		2.20	20.50	1.63	60.75	22.88
SD		1.40	1.35	0.04	7.03	2.32
Cheer Team	C1	1	19	1.65	60.96	22.39
	C2	2	20	1.63	54.43	20.54
	C3	2	20	1.59	47.26	18.69
	C4	2	20	1.56	62.77	25.73
	C5	2	20	1.66	65.59	23.80
	C6	1	19	1.59	59.24	23.43
	C7	2	20	1.69	60.30	21.11
	C8	2	20	1.60	67.76	26.47
	C9	2	20	1.60	53.70	20.98
Mean		1.78	19.78	1.62	59.11	22.57
SD		0.44	0.44	0.04	6.39	2.53
Non-athlete Female Controls	N1	-	24	1.60	54.18	21.16
	N2	-	22	1.57	54.00	21.99
	N3	-	21	1.55	50.90	21.20
	N4	-	22	1.74	63.70	21.04
	N5	-	21	1.63	56.73	21.46
	N6	-	23	1.66	73.18	26.44
	N7	-	21	1.57	53.45	21.55
	N8	-	21	1.68	60.82	21.64
	N9	-	23	1.74	101.00	33.36
	N10	-	21	1.66	52.90	19.11
Mean		-	21.90	1.64	62.09	22.90
SD		-	1.10	0.07	15.21	4.11
p value		-	0.001	0.68	0.827	0.968

APPENDIX IV: T-TEST COMPARISONS

Table 6: T-test Comparisons

	#	Total Body BMD (g/cm^2)	Total Body Z-score	Spine BMD (g/cm^2)	Spine Z-Score	Dual Femur (g/cm^2)	Dual Femur Z-Score	Serum Vitamin D (ng/mL)	Daily Calcium Intake (mg)	Daily Vitamin D Intake (IU)
Dance Team	D1	1.25	1.60	1.52	2.20	1.23	0.60	26.82	994.00	261.00
	D2	1.21	1.00	1.33	1.10	1.09	0.10	24.62	99.00	202.00
	D3	1.30	2.10	1.39	1.60	1.23	1.00	33.40	28.00	29.00
	D4	1.37	2.10	1.62	2.70	1.27	0.20	22.71	2197.00	1079.00
	D5	1.11	-0.20	1.15	-0.40	0.98	-	23.36	76.00	146.00
	D6	1.20	1.30	1.41	1.70	1.18	-	25.99	1157.00	586.00
	D7	1.20	0.80	1.35	-	1.09	-	31.20	144.00	49.00
	D8	1.36	4.30	1.63	-	1.51	-	27.77	206.00	150.00
	D9	1.12	0.30	1.20	-	1.13	-	23.95	121.00	34.00
	D10	1.21	1.30	1.30	-	1.28	-	34.65	14.00	23.00
Mean		1.23	1.46	1.39	1.48	1.20	0.48	27.45	503.60	255.90
SD		0.09	1.23	0.16	1.07	0.14	0.41	4.26	723.55	335.07
Cheer Team	C1	1.19	1.10	1.32	1.00	1.13	-	35.63	490.00	480.00
	C2	1.15	0.30	1.16	-0.30	0.79	-1.80	22.79	725.00	382.00
	C3	1.19	0.90	1.31	0.90	1.15	1.00	17.17	281.00	154.00
	C4	1.13	0.10	1.34	1.10	1.04	0.20	22.52	297.00	125.00
	C5	1.42	-	1.68	-	1.38	-	17.29	628.00	385.00
	C6	1.16	0.50	1.21	0.10	1.06	-	20.91	357.00	111.00
	C7	1.24	1.70	1.54	-	1.13	-	38.96	1018.00	100.00
	C8	1.27	0.80	1.35	-	1.22	-	22.94	564.00	168.00
	C9	1.18	1.20	1.37	-	1.13	-	23.14	422.00	149.00
Mean		1.22	0.83	1.36	0.56	1.11	-0.20	24.59	531.33	228.22
SD		0.09	0.52	0.16	0.62	0.16	1.44	7.61	236.22	144.86
p value		0.70	0.19	0.72	0.12	0.23	0.40	0.32	0.91	0.82

APPENDIX V: ANOVA

Table 7: Total Body BMD (g/cm^2) ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.040	2	.020	2.175	.134
Within Groups	.237	26	.009		
Total	.277	28			

Table 8: Total Body BMD Z-Score ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.065	2	4.032	3.468	**.047**
Within Groups	29.068	25	1.163		
Total	37.133	27			

APPENDIX VI: MULTIPLE COMPARISONS

Table 9: Total Body BMD (g/cm^2) Multiple Comparison

Tukey HSD for TOTAL BODY BMD G/CM^2

(I) Sport		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Dance	Cheer	.01856	.04389	.907	-.0905	.1276
	Control	.08500	.04271	.135	-.0211	.1911
Cheer	Control	.06644	.04389	.301	-.0426	.1755

Table 10: Total Body BMD Z-Score Multiple Comparison

Tukey HSD for TOTAL BODY BMD Z-SCORES

(I) Sport		Mean Differenc e (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Dance	Cheer	.63500	.51148	.441	-.6390	1.9090
	Control	1.27000*	.48223	.037	.0689	2.4711
Cheer	Control	.63500	.51148	.441	-.6390	1.9090

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