ASSESSING LONGITUDINAL CHANGES IN BONE MINERAL DENSITY IN MALE AND FEMALE CROSS COUNTRY RUNNERS

BY

CARTER MCCANCE DENNE

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Approved By:

Peter H. Brubaker, Ph.D., Advisor

Gary D. Miller, Ph.D., Chair

Heath C. Thornton, M.D.

DEDICATION

This thesis is dedicated to all those who have helped me to this point in my journey through life. To my parents, Claire and Fred Denne, for their unconditional love and support. Your love has pushed me to be a better father, partner, and student. To my two beautiful children, Ella and Kailen, for showing me every day what life is really about. Your smiling faces are the light in my world. To my love Taylor for her support, friendship, and guidance in our many adventures. You are my happy thought.

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ABSTRACT

ASSESSING LONGITUDINAL CHANGES IN BONE MINERAL DENSITY IN MALE AND FEMALE CROSS COUNTRY RUNNERS

C. Denne, J. Ross, A. Bledsoe, N. Wooten, P. Brubaker FACSM. Dept. of Health and Exercise Science, Wake Forest University, Winston-Salem, NC 27109

The results of several recent research studies have questioned the benefit of high levels of running and its influence on bone mineral density. Elite endurance athletes often experience high physiological stress, hormonal imbalances, and nutritional inadequacies during competitive seasons as a result of training and competing. The effect of these factors on bone mineral density is not well understood. **Purpose**: To examine changes in body bone mineral density (BMD) in male and female collegiate cross country runners over the period of a competitive season (pre- post CC). **Methods:** Subjects were 23 (11 male and 12 female) Division I collegiate cross country runners ages 18-22 yr. BMD was measured using dual x-ray absorptiometry (DXA) by the GE Lunar iDXA Bone Densitometer. BMD was measured in the total body, spine, hips, and non-dominant forearm. Body fat percentages were measured using DXA and standardized skin fold measures. Maximal oxygen uptake (VO_2 max) was measured using a maximum treadmill test. Caloric intake was calculated from a 7-day food log and running mileage quantified using self-reported training logs. **Results:** Due to lack of adherence to study requirements only 8 males and 8 females were evaluated for changes from pre-post CC. As expected, at pre- CC males were taller, heavier, running more miles, and had a higher VO_2 maximum than females. From pre- to post CC season males did not have any changes in body composition whereas females had an increase of 2.7 ± 1.7 lbs or 2.1% of total mass.

From pre- to post season males did not have any significant changes in BMD whereas females had a significant decrease in BMD of L1, L1-2, and L1-3 vertebrae. As a group, males and females did not have any BMD value that significantly differed from the reference population but 4 males and 4 females had total BMD that were lower than "normal". At pre- CC male total body BMD and forearm BMD positively correlated with lean body mass whereas hip BMD negatively correlated with running mileage. At pre- CC female hip BMD was negatively correlated with % body fat. From pre- to post CC males showed significant decreases in vitamin D levels whereas females showed significant increases in iron and magnesium levels. **Conclusion:** Despite limitations, this study adds substantially to our understanding of BMD levels of competitive collegiate CC runners. The results of this study suggest that a competitive CC season has minimal negative or positive impact on BMD on collegiate CC athletes. However, approximately 50 % of the male and female athletes in this study have BMD levels below their age-matched normal levels. The low BMD levels seen in these distance runners may increase their risk of stress fractures and/or osteoporosis later in life. Further investigation of this important topic is warranted and should be performed on a larger and more diverse group of collegiate distance runners.

REVIEW OF THE LITERATURE

Functions and Properties of Bone

Bone is a connective tissue that serves several functions in the human body, including; protecting internal organs, providing structure in the body, providing levers for motion, transducing sound in the ear, producing red blood cells, storing minerals, maintaining pH balance with acid buffering, and storing fat. Like all tissues of the body, bone is living and continually changing.

There are both organic and inorganic substances that make-up bone tissue. The inorganic portion of bone is composed of minerals and salts. These minerals and salts are found primarily in the matrix of the bone. This matrix is the hard portion of the bone made from hydroxyapatite which is a conglomerate of phosphate, calcium, and hydroxide. The organic portion of bone includes blood vessels, nerves, collagen, and cells. Collagen is a protein that increases the flexibility and structural strength of bone[1]. Collagen fibers run throughout the bone in the matrix. The two primary cells of the bone are osteoblasts and osteoclasts. The primary role of osteoblasts is to deposit new bone material to maintain and increase bone material[2]. Osteoclasts work in the opposite manor by breaking down bone material[3].

A bone is composed of two layers with different types of bone material. The outer layer is composed of cortical bone and the inner layer is composed of trabecular bone.

Cortical bone is more dense and composed of compact bone matrix. The cortical bone provides structural strength to the bone to withstand the forces that are placed on the bone. Trabecular bone is less dense, more porous, and is metabolically active containing red bone marrow for hematopoiesis[4].

Bone accrual and loss is affected by both the age and gender of the individual. Ferretti (1998) studied 778 girls and boys (ages 2–20 years) as well as 672 men and women (ages 20–87 years) and demonstrated significantly age and sex differences. It was shown that both men and women stored more mineral per unit of lean body mass within the reproductive period than before puberty (13%–29% and 33%–58%, respectively). Additionally, it was shown that women stored more mineral than age-matched men with comparable lean body mass (17%–29%) until menopause. Also postmenopausal women had lower values of bone mineral than premenopausal women. Men showed no age effect on the total bone mineral content to lean body mass relationship after puberty[5].

Unhealthy Bone

Osteoporosis is characterized as a progressive bone disease in which there is a decrease in bone mass and density leading to an increased risk of fracture. Bone is lost when osteoclast activity surpasses the activity of the osteoblasts resulting in a net loss of bone[6]. Bone loss is a natural process that occurs with aging [7]. Modern medicine and technological advancements have increased the lifespan of the population allowing individuals to live longer. As a result, there is an aging population which has a reduced bone mass leading to an increase in the prevalence of individuals with osteoporosis.

Osteoporosis has become a worldwide health issue affecting older adults in all parts of the world. Using the World Health Organization (WHO) guidelines, osteoporosis is diagnosed when bone mineral density (BMD) is more than 2.5 standard deviations below the peak bone mass reference standard for young women. When these criteria are applied to women in the United States, it is establish that there would be approximately 5 million women diagnosed with osteoporosis. If the WHO guideline for osteopenia (1-2.5 standard deviations below peak bone mass) were applied, an additional 13–17 million women would be estimated to have this disorder. Using the same criteria for men, approximately 1–2 million men would be diagnosed with osteoporosis and another 8–13 million would be diagnosed with osteopenia[8,9].

Individuals with osteoporosis and/or osteopenia are at an increased risk for fracture in the wrist, foot, humerus, hip, rib, toe, leg, pelvis, hand, and clavicle[10]. Fractures in older adults have resulted in a great economic burden on our healthcare system. It is estimated that by 2025, fractures are projected to increase by >48% to >3 million fractures, resulting in $25.3 billion in healthcare costs. The cumulative cost of incident fractures is expected to increase from $209 billion from 2006 to 2015 to $228 billion for 2016–2025. From 2006–2015 the annual fracture incidence is projected to rise by 22%, whereas annual costs are projected to increase >20% [11].

Risk Factors of Osteoporosis

The reduction of bone mass in the body is expected during the normal aging process. There are, however, specific factors that have been shown to accelerate bone loss in aging individuals. Longitudinal studies have examined the relationship between

bone mineral density and age, weight, change in weight, height, smoking, caffeine, alcohol use, physical activity, serum 25-OH vitamin D, calcium intake, and current estrogen replacement for women. A study by Hannan et al.[12], in 2000 investigated the bone loss of older adults (mean age 74 years) over a four year period. Hannan et al., found that BMD decreased over the 4 year period in both elderly women and men. Specifically, the average four year BMD loss for women (range, 3.4 – 4.8%) was greater than the loss for men (range, 0.2–3.6%) at all bone sites. For women, there was an association between BMD decrease and lower baseline weight, weight loss in interim, and amount of alcohol use. Conversely, women who gained weight during the interim gained or had little change in BMD. For women, the use of estrogen replacement had a protective affect against BMD loss. In men, lower baseline weight and weight loss were also associated with BMD loss. Men who smoked cigarettes at baseline lost more BMD at the trochanter site than those who did not smoke. Interestingly, there was no association between bone loss and caffeine use, physical activity, serum 25-OH vitamin D, or calcium intake [12].

Younger adults can also be susceptible to a loss of bone. Abnormal bone loss is usually associated with poor diet, high alcohol consumption, cigarette smoking, excessive physical activity, absent or irregular menstruation, low body weight and body fat in younger adults[13,14].

Stress Fractures

A stress fracture can occur when the forces on the bone are greater than the bones ability to withstand and respond to these forces[15]. A stress fracture is an incomplete fracture that results from the repeated loading of a weight bearing bone [16]. Athletes are

highly susceptible to stress fractures because their bones experience excessive forces on a regular basis during training and competition. There are currently 1,000 National Collegiate Athletic Association (NCAA) affiliated schools with an estimated 420,000 collegiate athletes in the United States [17]. A retrospective study of a single Division I institution found a high incidence of stress fractures among athletes. Over a 10 year period there were 74 confirmed cases of stress fractures [18]. If this average of 7.4 stress fractures per year were consistent among all NCAA affiliated schools, there would be an estimated 7,400 stress fractures per year or 74,000 stress fractures over a 10 year period. Long distances runners experience high forces on their bones due to large volumes of running and are therefore more likely to sustain a stress fracture than other athletes [19].

Not only are collegiate runners and athletes affected by stress fractures but so are the general public, specifically those who run regularly. Roughly 7.1% of Americans, 18 years of age or older, take part in running as a form of exercise and recreation. Of these runners, up to 20% will suffer from a stress fracture at least once in their lifetime[15]. Thus, stress fractures are a significant issue for athletes and the general population that run regularly.

Several risk factors identified have been associated with the development of stress fractures. Including irregular menstruation, low bone mineral density, low lean mass, nutritional deficiencies, and low physical fitness[20].

Quantifying Bone Health

Bone health is assessed primarily by quantifying BMD and bone mineral content (BMC). Bone mineral density refers to the amount of mineral matter per square

centimeter of bone. Bone mineral content can be calculated by the ratio of weight to the volume or area of the bones. The greater the bone mineral density, the greater the strength of the bone[21]. Clinically, BMD and BMC can be used as indirect indicators of fracture risk or the risk of developing osteoporosis. However for athletes, the relationship between BMD or BMC with stress fractures or osteoporosis is not conclusive. According to a study by Myburgh[22] (1990) athletes with a lower BMD had a higher incidence rate of stress fractures. Iwamoto[23] (2011) also found an association between low BMD and an increased risk for stress fracture. However, Duckham[24] (2012) found that BMD was not a significantly related to stress fractures in young female runners. Generally, athletes show an increased BMC later in life, suggesting that exercise and/or sport have a positive effect on bone health and reduce the risk of osteoporosis and other bone disorders[25]. The focus of these studies has been on the female athlete and little attention has been placed on male athletes, especially male endurance athletes. The studies on male athletes are typically cross-sectional with limited longitudinal investigations. Assessment of the limited number of studies on male athletes suggests that males also lose BMC and BMD with vigorous physical activity in a similar to female athletes[26,27].

There are several ways to measure BMD and BMC such as dual-energy x-ray absorptiometry (DXA or DEXA), quantitative computed tomography (QCT), qualitative ultrasound (QUS), single photon absorptiometry (SPA), dual photon absorptiometry (DPA), digital x-ray radiogrammetry (DXR), and single energy x-ray absorptiometry (SEXA). Of these, DXA is the most commonly used in the clinical assessment of bone health. The other techniques are used primarily in research settings [28].

Dual-energy x-ray absorptiometry (DXA) is a common technique used to assess body composition because it is non-invasive, safe, and cost effective. Contemporary DXA scanners are able to accurately measure total fat mass, percent body fat, total lean mass, total BMD, and total BMC. These DXA scanners are also able to measure fat mass, lean mass, and BMC in regional areas including the arms, legs, trunk, gynoid, and android locations. Bone mineral density of the forearm, lumbar spine, and hips can be measured as well. Dual energy x-ray absorptiometry has been shown to be a valid technique for measuring fat-free mass and lean mass as it is highly correlated ($R^2 = 0.98$) to multislice computed tomography (CT)[29]. Additionally, DXA has been shown to be a reliable measure for BMC ($r = 0.99$), lean mass ($r = 0.99$), fat tissue mass ($r = 1.00$), and bone mineral density ($r = 0.98$)[30].

Factors Affecting Bone Health

Bone is a living tissue that is constantly remodeling and changing its make-up. These changes influence the properties of the bone, including the mineral density. There have been several studied factors that affect bone and its mineral density. Known factors that affect bone include body composition, type of exercise, volume of exercise, changes in hormones and hormone statues, and dietary factors.

Body Composition

Both fat and lean mass have been shown to influence BMD and bone properties. A higher amount of lean muscle tissue has been correlated with a higher BMD[31,32].

Internal forces generated during muscle contractions increase the stress on tendons which then apply stress to bone. A greater amount of muscle mass generates greater internal forces, causing higher forces on the bone and influences BMD in a dose response fashion [33]. Lean mass and other properties of muscle, such as strength and power, have been positively associated with BMD. Witzke and Snow[32] determined that in adolescent girls, lean body mass and leg power were strong predictors of BMD for the whole body, lumbar spine, femoral shaft, and hip.

Total fat mass may also effect BMD through several proposed mechanisms including increased mechanical loading of the bone, estrogen production, and serum insulin modulation[34, 35, 36]. Additionally, fat mass appears to affect BMD differently in males and females. The influence of fat and lean mass in adolescent children ages (14-16 years old) were investigated in a study by Hage et al.[37], 2009. In adolescent females, both lean mass and fat mass were positively correlated with whole body BMD. In adolescent females, fat mass appears to be a stronger predictor of BMD than lean mass. In contrast, lean mass was a better predictor of BMD than fat mass in adolescent boys. Furthermore, fat mass was negatively associated with BMD in boys. The positive effect of fat mass on BMD in females is most likely due to hormonal factors that are not relevant in males[38].

Exercise and Physical Activity

The modality of exercise appears to be a highly influential factor on BMD levels in athletes[39] . Weight bearing exercises are known to have the most positive effect on

bone due to the ground reaction forces (external forces) and muscle forces (internal forces) that are placed on the bone[19]. These forces stimulate osteoblasts to produce bone matrix thereby increasing the bone density[40]. Weight bearing exercises involve the loading of the long bones of the body including jumping, running, dancing, stair climbing, aerobics, walking, and skiing. Taaffe[40] (1997) found that female gymnasts and runners had higher BMD then sedentary college students, primarily due to the ground impact that occurred during the participation of these sports. These finding are consistent with results of other investigations that have shown an increase in BMD levels in weight bearing athletes[25, 41, 39]. In children, the amount of weight bearing activity has been shown to be highly positively correlated with BMD[39].

Alternatively, the lack of weight bearing activity is associated with a decreased BMD in both athletes and sedentary controls[42, 43]. Non-weight bearing activities are those that do not load the long bones and typically include a device or fluid that supports the body's weight. Non-weight bearing activities include cycling, swimming, water aerobics, and seated rowing. A study by Rector and colleagues[42] in 2008 determined that male cyclists had lower whole body and lumbar spine BMD when compared with weight bearing athletes. Additionally, the non-weight bearing male athletes were seven times more likely to have osteopenia than the weight bearing male athletes. Penteado and colleagues[43] also demonstrated the lack of osteogenic effects with cycling. When compared to sedentary individuals, cyclists had significantly lower BMD measures.

The volume of exercise has also been shown to correlate with changes in BMD. A high volume of exercise has been associated with low bone mineral density in runners. MacDougall[44] (1992) determined that runners with a weekly mileage of 15-20 miles had

higher BMD than those who ran more or less. Runners who ran 60-75 miles per week demonstrated non-significant lower BMD levels compared to the sedentary controls. Burrows and colleagues[45] also found a similar relationship in women. As running mileage increased there was a significant reduction in both lumbar spine and femoral neck BMD. The possible mechanisms for these reductions in BMD are not fully understood due to the multifactorial influences on bone, but a possible mechanism for the decreased BMD may be related to ground reaction forces. Cumulative ground reaction forces can create excessive stress with high running volumes, leading to a decrease in BMD over time.

Hormones

The production of sex steroids at the onset of puberty are clearly linked with an increase of bone mineral acquisition during this period. Both adolescent boys and girls experience an increase in bone size resulting from an enlargement of the outer diameter of bone and a widening of the medullary diameter[46]. Hormones such as testosterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) play a major role in the deposition, maintenance, and degradation of the skeleton. Therefore, changes in hormones may have a profound effect on bone tissue in distance runners[47].

Testosterone is produced in the testes of males and estrogen and estrogen is produced in the ovaries of females. Both hormones play an important role in bone formation and maintenance in both males and females[48]. Rather than acting directly on the bone, testosterone is aromatized in the body to form estradiol which is the primary

form of estrogen in the human body[49]. The major physiological effect of estrodiol is to inhibit bone resorption and promote bone formation[50]. Estrogens affect bone by promoting osteoclast apoptosis which decreases the lifespan of the osteoclasts within the bone[51]. Conversely, osteoblast activity is increased with estrogen exposure which in turn promotes the formation of new bone matrix[52]. With age, the amount of testosterone and estrogen in both males and females decreases[53, 54]. Consequently, a reduction in bone mass is due to the reduced protective mechanisms of the hormones[55] .

Also important to bone health are gonadotropins which include luteinizing hormone (LH) and follicle stimulating hormone (FSH). Luteinizing hormone is produced in the anterior pituitary gland and affects the gonads. In males, LH causes the Leydig cells of the testes to produce testosterone. In females, LH causes the Theca cells in the ovary to respond by secreting testosterone. The testosterone is then converted into estrogen by adjacent granulosa cells. Additionally, LH causes ovulation of a mature follicle in females. Like LH, FSH is produced in the anterior pituitary and acts to improve the function of LH. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. In women, the reduction of both LH and FSH have been shown to negatively influence BMD[56].

Male distance runners commonly experience a reduction in testosterone associated with training volume and/or competition[57, 58, 59]. Running mileage in males has also been shown to be negatively correlated with testosterone levels[60]. In contrast, elite male weight lifters experience an increase in serum testosterone with training over long durations[61]. However, another study from the same group showed that serum testosterone did not change significantly in a different populations of elite male weight lifters[62]. No

change in serum testosterone levels was reported in elite male swimmers during competitive training[63]. Study of elite male triathletes, elite male cyclists, and recreational male marathon runners showed no significant changes in LH, FSH, and serum testosterone over the period of a competitive year. Female runners do not typically experience a reduction in testosterone during training, but often experience a reduction in LH, FSH, and estrogen levels[64, 65, 59]. One study demonstrated that testosterone increases in adolescent females competing in long distance competition[66], but another study found that females engaging in weight training did not experience a change in serum testosterone[67]. This variability in the changes in testosterone levels of females may be attributed to the population investigated, the mode of exercise, as well as the design of the studies.

The mechanism responsible for the change in hormone levels from distance running is likely due to a decrease in the release of gonadotropins, which include LH and FSH. The decrease in gonadotropins is primarily due to the reduction of gonadotropin-releasing hormone (GnRH) produced by the hypothalamus[68]. Gonadotropin-releasing hormone is responsible for triggering the release of LH and FSH from the anterior pituitary which results in the release of sex hormones from the gonads[69]. The relationship between the hypothalamus, the pituitary gland, and the subsequent effect on the gonads, creates what is known as the hypothalamic-pituitary-gonadal axis[70]. Therefore, low levels of estrogen and testosterone can be caused by the reduction of GnRH and create a condition of hypogonadism. Hypogonadism is a deficiency of the gonads to produce sex hormones which affects pubertal maturation and reproductive function. This condition has been associated with a reduction of the BMD in women[65, 71, 72]. The changes in LH

and FSH and their influence on testosterone and BMD has not been established in male athletes.

The Female Athlete Triad (FAT), first described by the American College of Sports Medicine (ACSM) in 1993, is a syndrome in which females demonstrate an interrelationship among energy availability, menstrual function, and BMD[73].

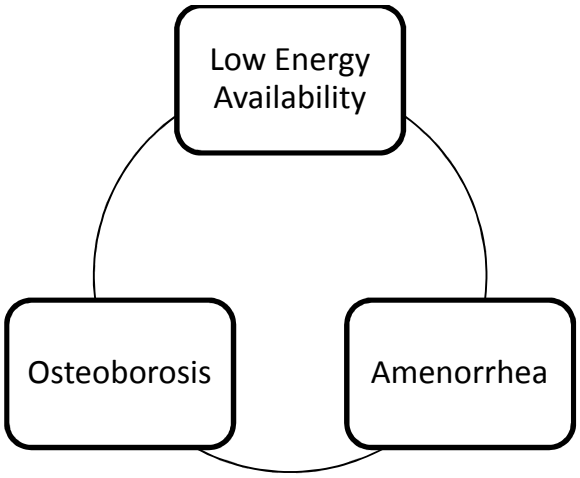


Figure 1: Graphical Representation of Female Athlete Triad

In 2007 the ACSM concluded that the Female Athlete Triad is initiation by a low "energy availability" state[73]. Energy availability (EA) can be defined as dietary energy intake, calories consumed or energy intake (EI), minus calories energy expenditure (EE). This can be reported in a relative form by normalizing it by fat free mass (FFM)[74]. This relationship of factors can be seen in the following equation.

$$EA = EI - EEE / (FFM), \text{ with a unit of kcals/ kg FFM}$$

A decrease in energy availability can result from a high expenditure of calories with physical activity, a low intake of calories, or a combination of both. Persistently low

energy availability can cause menstrual dysfunction and changes to the estrogen levels of females[75]. The resulting low estrogen levels may have a profound effect on BMD, depending on the degree of disruption to the menstrual cycle[76, 45].

Recent research has also demonstrated that male endurance athletes can suffer from a similar condition, described as the male endurance athlete tetrad (MEAT)[74]. MEAT is comprised of the interrelationships between energy availability, bone density, testosterone levels, and cardiovascular health.

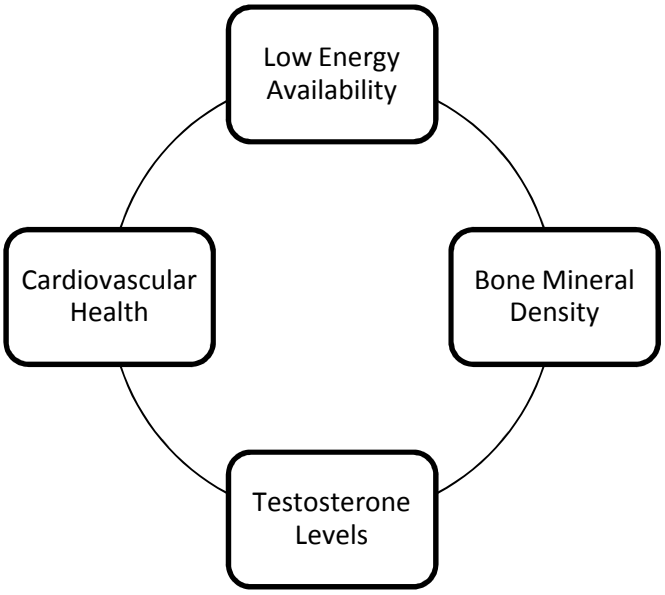


Figure 2: Graphical Representation of the Male Endurance Athlete Tetrad

Male endurance runners engaging in a high volume of training or a low energy intake can have low energy availability. As previously discussed, male runners have shown a reduction in testosterone which has been shown to be positively associated with running mileage[57, 58, 59]. The low energy availability and reduction in testosterone are believed to cause a reduction in BMD, specifically in the radius and lumbar spine[77]. Limited research has also shown that males who participate in competitive distance running have low

density lipoprotein cholesterol (LDL-C) and triglycerides levels that are higher than recommended values[78]. High LDL-C and triglycerides are strong risk factors for cardiovascular disease[79]. A diet high in carbohydrates may cause the increase in triglycerides and LDL-C[80, 81].

Dietary Factors

Dietary factors play a complex and important role in bone health. The intake of macronutrients, micronutrients, and calories has an effect on the development, repair, and maintenance of all tissues in the body, particularly bone. Macronutrients include protein, fat, and carbohydrates that contain calories which can be metabolized for energy. Micronutrients include vitamins, minerals, antioxidants, and phytochemicals that do not contribute calories for metabolism. Macro and micronutrients effect bone tissue in a complex, synergistic, and multifactorial way.

Higher amounts of protein in the diet have been correlated with total body BMD[82]. Radius and spine BMD has also been shown to correlate with protein intake[83]. Fat intake has also been shown to be inversely related to BMD. Saturated fats have been shown to be inversely correlated with BMD of the femoral neck[84]. Macdonald and colleagues[85] found that a high intake of unsaturated fatty acids are associated with BMD reduction in the femoral neck[85]. Presently there is a lack of scientific studies investigating the effect of carbohydrate's influence on BMD to make a conclusive statement on the role of this macronutrient in bone health.

Because bone is composed of many minerals and micronutrients components including calcium and phosphorus, it is important to ingest these nutrients to maintain and improve bone mass [86]. Adequate intake of calcium is associated with higher BMD in the lumbar spine, femoral neck, femoral shaft, and total body[87, 88, 89]. Diets high in fruit and fiber intake have also been shown to positively affect BMD[90, 82, 85]. Furthermore, higher BMD are also associated with a higher intake of zinc, magnesium, potassium, and fiber[91]. Reduced intake of potassium, β-carotene, magnesium, vitamin C, and vitamin K have been associated with increased bone reabsorption and a decrease in BMD and an increase in fracture risk [90, 92, 93].

Dietary patterns have been used to create a more complete picture of the interactions between macronutrients and micronutrients and BMD. McNaughton and colleagues[94] identified two dietary patterns that influenced BMD. A high consumption of refined cereals, soft drinks, fried potatoes, sausages and processed meat, vegetable oils, beer, and takeaway foods along with low consumption of other vegetables, vegetable dishes, tea, coffee, fruit, wholegrain breads, and breakfast cereals is strongly associated with a decreased total body BMD. Alternatively, a high consumption of legumes, seafood, seeds, nuts, wine, rice and rice dishes, other vegetables and vegetable dishes along with a low consumption of bacon and ham was associated with increased BMD at the femoral neck and spine[94]. Similarly, Zallaua and colleagues identified that a diet high in dairy and low in fat was associated in gains in hip BMD. Similarly a diet high in fruits, vegetables, and fiber intake and low in fat was associated with improvements in total body BMD[82].

Summary and Rational For This Study

Aims and Hypotheses

Osteoporosis and bone related disorders represent a significant issue for an aging population. The body of literature identifies physical activity and exercise as prevention to reduce the risk of osteoporosis and fracture later in life. However, bone health in young male and female adults engaging in high amounts of exercise has not been adequately studied. This is primarily due to a lack of longitudinal studies that identify the change in bone mineral density in both female and male distance runners. The first aim of this study is to identify the differences between male and female distance runners at baseline as well as to population based normative values. It is hypothesized that male distance runners will have significantly higher levels of BMD then female distance runners. Furthermore, male long distance runners will have higher BMD compared to the population based norms, whereas female distance runners will be lower than population norms. The second aim of this investigation is to observe the change BMD and body composition from pre- post Cross-Country season in male and female distance runners. It is hypothesized that total body bone mineral density will not change, non-dominant forearm bone mineral density will decrease, and both hip/femur and lumbar spine bone mineral density will increase from pre- to post. The final aim of this study is to identify baseline factors such as caloric intake, running mileage per week, lean mass, percent body fat and testosterone that correlate with BMD levels in male and female distance runners.

METHODS

Participants

Subjects for this study were recruited from the Wake Forest University NCAA cross country team. Twenty three (11 male and 12 female) Division I collegiate cross country runners 18-22 years of age agreed to participate. These individuals met the following inclusion criteria: over the age of 18 years old, a member of the team, not currently injured, currently training, willing and able to participate in all aspects of the trial, and were able to provide written consent approved by the institutional review board. Participants were excluded from pre- post analyses if they did not complete both baseline and follow-up measures within the one week window of participant testing and/or follow-up measures were made more than two weeks after last competition or expected training date.

Measures

Body Composition Assessment: DXA

A DXA scan was performed on participants at baseline and follow-up using the GE Lunar iDXA Bone Densitometer. Separate DXA measurements were taken of the total body, anterior-posterior (AP) lumbar spine, non-dominant forearm, and dual femoral neck. The total body scan was done with the patient lying supine with arms and legs fully extended. Arms were placed by their sides with hands placed vertically on the scan table with the thumbs and fingers held in alignment. The feet were held vertical by placing a strap around the ankles.

The total body scan provided data on total body mass, total fat mass, percent body fat, total lean mass, total BMD, and total bone mineral content (BMC). Additionally, the total body scan provided regional estimates of arm fat mass, leg fat mass, trunk fat mass, android fat mass, gynoid fat mass, arm lean mass, leg lean mass, trunk lean mass, andriod lean mass, and gynoid lean mass. Regional sites were measured by the GE iDXA software. The arm region is comprised of the arm and shoulder which is started at the glenohumeral joint with a diagonal line through the axillary crease. The trunk region includes the neck, chest, abdominal and pelvic areas. Borders are at the inferior edge of the chin and extend down to the inferior portion of the pelvis to then dissect the femoral neck to include the upper portion of the femoral necks and the femoral heads. The leg region includes all of the area below the lines that form the lower borders of the trunk including the pelvis. The android region is the area below the ribs and extends to half way between the iliac crest and pubic symphysis of the pelvis. The gynoid region includes the hips and upper thighs, and extends from the lower border of the android region with the lower border at the superior one third of the femur. The lumbar spine scan provided data for the BMD of L1, L2, L3, L4, L1-2, L1-3, L1-4, L2-3, L2-4, and L3-4. Remaining in the same position, each femur was then scanned. A re-scan was done if the lesser trochanter was indicated as prominent by the software. Bone mineral density of the femoral necks were provided at the femur neck mean, upper neck mean, lower neck mean, Ward's Triangle mean, trochanter mean, and total hip/femur mean. The non-dominant forearm was scanned by internally rotating the shoulder so the forearm is prone and parallel to the scan table. An alignment board was then placed under the forearm to align the forearm in a line parallel with the table. Bone mineral density of the non-dominant

forearm were given at the ultra-distal radius, ultra-distal ulna, ultra-distal ulna and radius, 33 % of radius length, 33 % of ulna length, 33 % of radius and ulna length, radius total, ulna total, and radius and ulna total.

Body Composition Assessment: Skinfolds and Circumferences

Body fat percentage was also estimated for each participant using skin-fold measures. An experienced clinical exercise physiologist obtained skin-fold measures at baseline and follow-up within 30 minutes before or after the DXA was performed. The skinfolds were taken on the right side of the body using the hand held Harpenden skinfold caliper. Three sites were taken on the females which included the suprailiac, tricep, and front thigh fold. The suprailiac fold was taken at the intersection of a line joining the spinal and anterior part of the axilla and a horizontal line at the level of the iliac crest. The pinch was taken toward the midline directed medially and downward, following the natural fold of the skin. The tricep fold was taken at the level of the mid-point between the acromion process and the olecranon process on the mid-line of the posterior surface of the arm. A vertical pinch, parallel to the long axis of the arm, is made at the tricep landmark. The front thigh fold was taken at the mid-point between the top of the patella and the inguinal fold on the anterior surface of the thigh. This was also a vertical pinch parallel to the midline of the body at the landmark. Seven sites were taken on the males which included the suprailiac, tricep, front thigh, axilla, chest, subscapular, and abdominal fold. A vertical fold was taken at the axilla. This fold was taken in line with the frontal plane at the level of the xiphoid process. A chest fold was taken at one-half the distance between the anterior axillary line and the nipple on a diagonal fold. A subscapular fold was taken at the lower angle of the scapula with a pinch along the

diagonal angle of the scapula. The abdominal fold was taken 5 cm lateral to the umbilicus at the level of the umbilicus with a vertical fold parallel to the midline of the body. Each site was taken then re-taken after all sites had been measured. If two measurements at a site were greater than 10% different, a third measurement was taken on that site. The two closest measurement values at a site were then averaged and used to determine body composition.

The Jackson and Pollock 3-site formula was used for females and the Jackson and Pollock 7-site formula was used for males to calculate body density [95] Jackson and Pollock, 1978. Body density was then converted to percent body fat using the Siri equation [96].

Jackson and Pollock Equations:

Woman 3-site (triceps, suprailiac, thigh):

Body density = 1.099421 – 0.0009929 (sum of three skinfolds) + 0.0000023 (sum of three skinfolds)^2 - 0.0001392 (age)

Men 7-site (chest, midaxillary, triceps, subscapular, abdomen, suprailiac, thigh):

Body density = 1.112 – 0.00043499 (sum of the seven skinfolds) + 0.00000055 (sum of the seven skinfolds)^2 – 0.00028826 (age)

Siri Equation:

Percent body fat = [(4.95/body density) – 4.50] X 100

Waist, hip, and thigh circumferences were also obtained. The waist circumference was taken at the narrowest part of the abdomen. The hip circumference was taken at the widest portion around the gluteals. The thigh measurement was taken half way between the inguinal fold and the top of the patella on the right thigh. Each circumference was obtained and then repeated after all circumferences had been measured. If two circumferences at a site defined by $\geq$ 10%, a third measurement was obtained at that site. The two closest circumferences at a site were then averaged and used as the final measurement.

Dietary Analysis

A diet history questionnaire (see Appendix A) was given during the baseline assessment to evaluate participant's dietary patterns. This questionnaire asked specifically about the use of supplements, specific diets followed, as well as the consumption of alcoholic and/or caffeinated beverages.

Participants were also asked to complete two 7-day food diaries (see Appendix B) to record all food and drink items consumed each day. The first food log was completed during the week of the baseline assessments (pre-CC). The second food log was completed the week prior to the follow-up assessments (post CC). The nutritional intake of three days out of the seven was used in the analysis. Two weekdays and one weekend day were analyzed using Nutritionist Pro version 5.2 software. The three day assessment was used to calculated the average daily caloric intake, average daily carbohydrate intake (% of total), average daily fat intake (% of total), average daily protein intake (% of total), average daily alcohol intake (% of total), average daily fiber intake, average daily

saturated fat intake, average daily mono unsaturated fat, average daily polyunsaturated fat, and average daily cholesterol intake.

Running History

At baseline, a running history questionnaire (see appendix C) was also given to each subject. This questionnaire inquired about participants running experience, current training status, and history of bone injury including stress fractures. Participant's running experience was established by inquiring about the number of years competitively running and personal records. Current training status was assessed by calculating weekly mileage for the past week, month, and three months. Participants were also asked to report any recent races and times. Past bone injury history including stress fractures, were obtained as well as how it was diagnosed.

As an additional method to analyze running mileage was a training log (see Appendix D) that was given to each participant. Each log was to be filled out for the duration of one month. These logs were given at baseline and again half way through the competitive season. Participants were instructed to record, in detail, their daily training and to record times, distance, and any other pertinent training information.

Female Athlete Questionnaire

Female participants were given an additional female questionnaire (see Appendix E) given at baseline. This female specific questionnaire was given to assess the female's menstrual history, menstrual status, and birth control use.

Follow-up Questionnaire

A condensed version of the baseline questionnaires (see Appendix F) was given as a single questionnaire at the follow-up visit. This questionnaire inquired about the competitive season, training status, injury occurrence, nutritional supplement use, caffeinated and/or alcohol intake, and a female specific portion. Participants were asked if they had competed this season and, if so, to list their times for the distances they competed in. In addition, they were asked about their mileage for the past; week, month, and three months. Participants were also asked if they had suffered any injuries this season and, if so, what was the injury. This question was followed by asking what was the last date of training or competition. Nutritional supplement use was determined by their questionnaire. Females then answered an additional portion about their menstrual cycle. Females were asked if they have had their menstrual cycle this month and, if so, what was the last date of ovulation and menstruation. Females were also asked how many times in the last three months did they have their menstrual cycle as well as about the current and past use of birth control medication.

Maximal Oxygen Uptake Analysis

Maximal oxygen uptake (VO_2 max) was measured using the Parvomedics TrueOne 2400 metabolic cart and software during a maximal graded exercise test on a treadmill. Subjects were asked to run on the treadmill to "warm-up" at a comfortable pace until they felt ready to begin the test. They were then fitted with headgear to hold a one way breathing valve and mouth piece secure in the participant's mouth. The mouth piece and one way valve allowed room air into the subject and the expired air directed it through a tube into the metabolic system. The test began at a running speed chosen by the

subject at a zero degree grade. The speed then remained constant throughout the entire test. During the test the grade increased 1.5 degrees every minute. Subjects were instructed to run until complete exhaustion at which point they were able to signal to stop the test or straddle the treadmill belt thereby ending the test. VO_2 max values were recorded as a relative value using body weight to standardize the volume of oxygen consumed given as milliliters per kilogram per minute (mL/kg/min).

Blood Analysis

Blood was drawn from each participant immediately before or after the DXA scan and before the completion of the maximal graded exercise test both pre- and post Cross-Country season. Participants had blood drawn at WFU Student Health Clinic. Approximately 8 mL (4 vials) of venous blood was drawn by a trained nurse at Wake Forest University Student Health. The vials of blood were centrifuged in the WFU Human Performance Laboratory for 10 minutes immediately after the blood was drawn. Within 24 hours the blood was transported to Laboratory Corporation of America (LabCorp) for the blood to be analyzed. . Blood analysis for luteinizing hormone, follicle stimulating hormone, testosterone, estrogen, vitamin D, iron, ferritin, calcium, and magnesium was performed. Results of the blood test were then faxed to Wake Forest University.

Statistical Analysis

All data was entered into SPSS (version 21) and analyzed for normality and demographics. Descriptive statistics were run at pre- and post Cross-Country season to determine means, standard deviations, and ranges for measured values. The data was found to not be normally distributed, thus, nonparametric statistics were employed to compare the groups. A Mann-Whitney U test was used to compare male and female values at pre- Cross-Country season. This method was also used to compare the pre-cross country season measures with normal reference values/ranges for males and females. A Wilcoxon Signed Rank test was used to compare pre- and post Cross-Country season values within subjects. Bivariate Spearman correlation analysis was used to examine associations between variables of interest. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Demographics

A total of 23 subjects, 11 males and 12 females, participated in this study. Descriptive statistics of demographic data pre- Cross-Country season (pre- CC) are presented in Table 1.

All participants were white non-Hispanic in race. Participants were between the ages of 18 to 22 years. As expected, males were significantly taller, had a higher BMI, and weighed more compared to females. Males also had a significantly higher weekly running mileage, greater running experience, and greater maximal oxygen uptake than females in this study. There were no differences in age between males and female subjects.

Of the 23 athletes that participated in the study, only 16 (8 males and 8 females) were analyzed for change in bone and body composition measures. Based on the exclusion criteria seven athletes were not included in these analyses. Three males were excluded because they were not re-tested within two weeks after last competition or expected training date. Four females were excluded; one due to dropout, two due to injury, and one was not re-tested within two weeks after last competition or expected training date.

Table 1: Demographics of Study Participants Pre- Cross-Country Season

	Male (n= 11)	**Female (n= 12)**
	Pre-CC	Pre-CC
Age (years)	19.5 ± 1.3	19.2 ± 1.3
Height (cm)	**180.0 ± 4.7**	**169.8 ± 5.5**
Weight (kg)	**67.0 ± 5.7**	**55.0 ± 5.5**
Body Mass Index	20.7 ± 1.2	19.0 ± 1.2
Running Experience (yrs)	**6.9 ± 3.0**	**5.0 ± 1.9**
Running Mileage (miles/week)	**65.9 ± 14.8**	**49.8 ± 20.5**
VO2 Max. (ml/kg/min)	**71.8 ± 4.4**	**59.9 ± 2.9**

- Data are presented as mean ± SD
- Data in bold indicates significantly (< .05) different between males and females Pre- CC

Study Measures

Dietary Intake Analysis

The average (± SD) dietary intake measures for baseline assessment (pre- CC) are presented in Table 2. Males had a significantly greater intake of calories, grams of protein, and cholesterol. There was no significant difference between males and females for percent carbohydrates intake, carbohydrate to body weight ratio, percent calories from fat, fat grams, percent calories from protein, and protein to body weight ratio. All other

dietary intake measures did not differ significantly from normal reference values/ranges at pre- CC.

Table 2: Dietary Measures Pre- Cross-Country Season and Normal Reference Values/Ranges

	Males (n= 7)	**Females (n= 5)**	**Normal**	
	Pre- CC	Pre- CC	Males	Females
Daily caloric intake (kcal/day)	**2638.9 ± 750.0**	**1947.9 ± 281.6**	NA	NA
Daily carbohydrate intake (% of total)	50.1 ± 4.9	49.3 ± 4.1	50-70[c]	50-70[c]
Carbohydrate intake to body weight per day (g/kg/day)	5.0 ± 1.9	4.4 ± 0.8	7-10[a]	7-10[a]
Daily fat intake (% of total)	30.5 ± 4.1	32.5 ± 3.9	20-35[a]	20-25[a]
Daily protein intake (% of total)	18.2 ± 3.5	18.2 ± 1.3	20-40[c]	20-40[c]
Protein intake to body weight per day (g/kg/day)	1.7 ± 0.3	1.6 ± 0.2	.8-1.2[c]	.8-1.2[c]
Daily cholesterol intake (mg/day)	**405.7 ± 149.2**	**179.4 ± 190.2**	NA	NA

- Data are presented as mean ± SD
- Data in bold indicates significantly (< .05) different between males and females Pre- CC
- NA = not available/applicable

(a) See reference [97]

(b) See reference [98]

(c) http://www.usada.org/files/active/athletes/NutritionBookletFinal.pdf

Total Body Composition Measures

The average (± SD) total body composition measures for pre- CC and post- CC are presented in Table 3 and Figures 2 and 3. There were no significant changes to the total body composition of males from pre-post CC, including total body mass, total fat mass, percent body fat (DXA and skinfold), total lean mass, total BMD, and total bone mineral content. Males did increase their lean body mass by 2.1 lbs (1.4%), but this increase did not reach statistical significance. On average the females significantly increased their lean body mass by 2.7 ± 1.7 lbs from pre to post CC, which is equivalent to an increase of 2.1 % of their total mass. There were no other significant changes in the total body composition measures in females.

Total body composition measures for males and females in this study did not differ significantly from normal reference values/ranges pre- CC or post CC.

Regional Body Composition Measures

The average (± SD) regional body composition measures for pre- post CC are presented in Table 4. As expected the males had higher lean mass in the upper and lower body sites, whereas they had lower fat mass in the upper and lower body sites compared to females. Males did not demonstrate any significant changes in regional body composition measures from pre-post CC season. However, they tended to increase their regional lean mass and decrease their regional fat mass regionally. The females experienced a significant increases in trunk, android, and gynoid lean mass (1.5 ± 1.0, .26

± .2, and .46 ± .4 lbs, respectively). Woman also had a significant decrease in thigh circumference of 1.1 ± .98 cm (2.1 %) but yet a trend to increase leg lean mass (p= .09).

Table 3: Total Body Composition Measures Pre and Post Cross-Country Season and Normal Reference Values

	Males (n= 8)		**Females (n= 8)**		**Normal**	
	Pre- CC	Post- CC	Pre- CC	Post- CC	Male	Female
Total Body Mass (lbs)	148.5 ± 9.3	149.2 ± 13.2	123.5 ± 11.1	125.1 ± 12.8	NA	NA
Total Fat Mass (lbs)	18.8 ± 3.0	17.4 ± 2.1	23.9 ± 4.1	22.9 ± 4.3	NA	NA
% Body Fat (DXA)	13.3 ± 2.1	12.2 ± .79	20.2 ± 2.9	19.1 ± 2.6	NA	NA
% Body Fat (skinfold)	7.0 ± 1.2	6.4 ± .6	16.2 ± 2.7	15.2 ± 2.5	NA	NA
Total Lean Mass (lbs)	123.1 ± 8.9	125.2 ± 11.0	**94.2 ± 9.1**	**96.9 ± 9.8**	NA	NA
Total Bone Mineral Density (g/cm^2)	1.24 ± .07	1.25 ± .07	1.15 ± .05	1.16 ± .05	1.22 ± .08[b]	1.18 ± .11[a]
Total Bone Mineral Content (lbs)	6.60 ± .64	6.64 ± .62	5.32 ± .53	5.37 ± .53	6.18 ± .93[c]	4.78 ± .62[c]

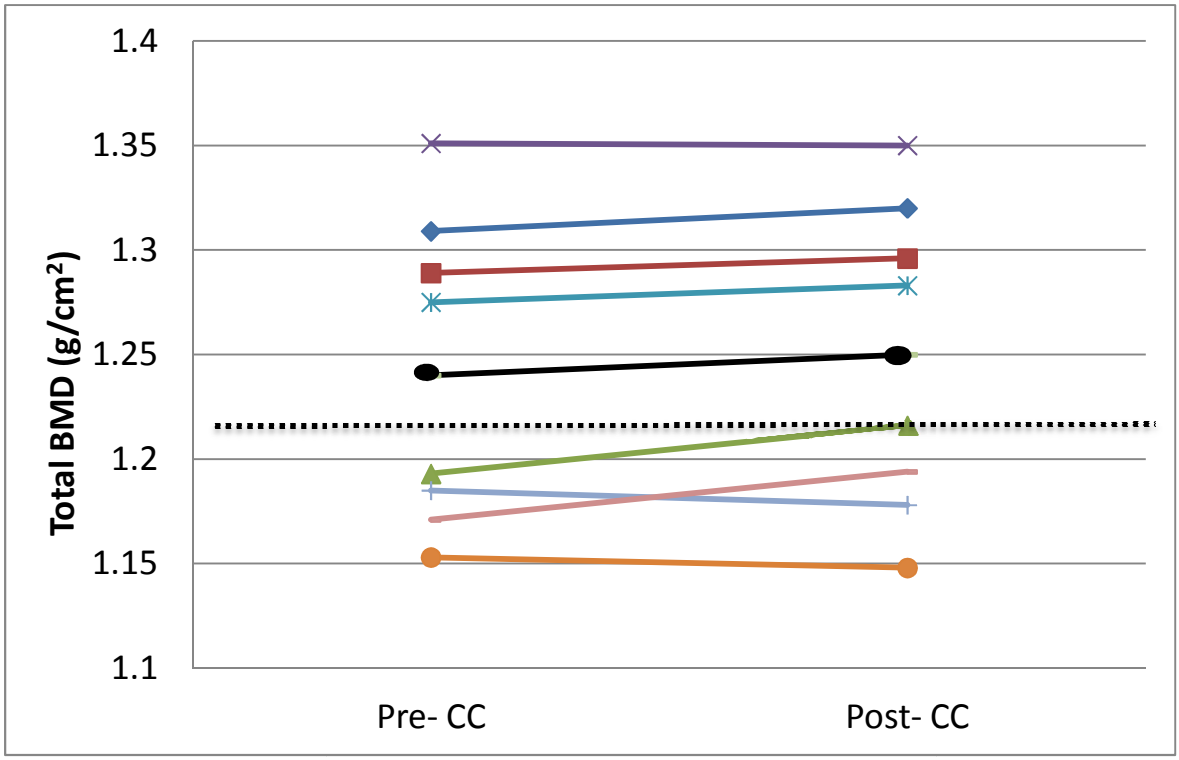
- Data are presented as mean ± SD
- Data in bold is significantly (p< .05) different from pre- to post
- NA = not available/applicable

(a) See reference [99]

(b) GE ilunar DXA reference

(c) See reference [100]

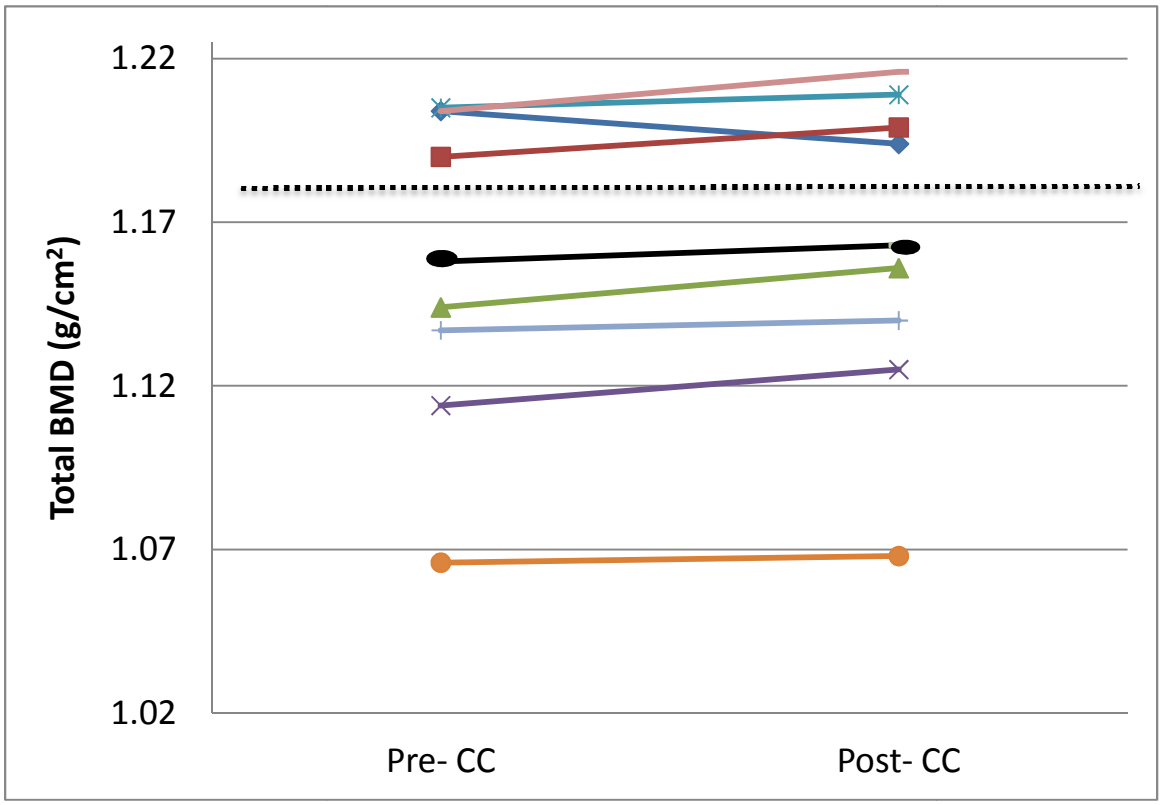
Figure 3: Total Bone Mineral Density of Individual Male Subjects Pre-Post Cross-Country Season



Represents Group Mean

Represents Reference Value (GE ilunar DXA reference)

Figure 4: Total Bone Mineral Density of Individual Female Subjects Pre-Post Cross-Country Season



Represents Group Mean

Represents Reference Value (see ref. [99])

Regional Bone Mineral Density Measures

Lumbar Spine

The average (± SD) lumbar spine BMD values for pre- post CC are presented in Table 6 and Figures 5 and 6. Males did not show any significant changes in lumbar spine BMD from pre- to post CC whereas females demonstrated significant decreases of .03

± .02 g/cm^2 in L1, .02 ± .03 g/cm^2 in L1-2, and .02 ± .02 g/cm^2 L1-3 BMD from pre to post CC, reflecting a decreases of 2.9 %, 2.2 %, and 1.6 % g/cm^2, respectively.

Despite the decrease in lumbar spine BMD in females, the values for males and females in this study did not differ significantly from normal reference data values/ranges pre- CC or post CC.

Forearm

The average (± SD) forearm BMD values pre- post CC are shown in Table 7. There were no significant changes in male forearm BMD from pre- post CC. Females also did not show any significant changes in forearm BMD. A trend was observed for a decrease in radius total (p= .06) pre- post CC in females.

Forearm BMD measures for males and females in this study did not differ significantly when compared to normal reference values/ranges pre- CC or post CC.

Table 4: Regional Body Composition Measures Pre and Post Cross-Country Season

	Males		**Females**	
	Pre- CC	Post- CC	Pre- CC	Post- CC
Arm Fat Mass (lbs)	2.0 ± .3	1.9 ± .2	3.0 ± .7	2.8 ± .6
Leg Fat Mass (lbs)	7.0 ± 1.2	6.4 ± .7	10.1 ± 2.4	9.9 ± 2.2
Trunk Fat Mass (lbs)	7.9 ± 1.9	7.2 ± 1.7	9.1 ± 1.4	8.4 ± 1.9
Android Fat Mass (lbs)	0.96 ± .23	0.86 ± .27	1.01± .19	.92 ± .29
Gynoid Fat Mass (lbs)	2.9 ± .7	2.7 ± .5	4.1 ± 1.1	4.7 ± 1.0
Arm Lean Mass (lbs)	14.6 ± 2.1	14.6 ± 2.0	9.1 ± .9	9.3 ± 1.1
Leg Lean Mass (lbs)	41.8 ± 3.5	42.3 ± 4.3	31.9 ± 3.7	32.6 ± 3.8
Trunk Lean Mass (lbs)	59.6 ± 3.6	60.9 ± 4.8	**46.7 ± 4.5**	**48.2 ± 5.2**
Andriod Lean Mass (lbs)	8.6 ± .8	8.7 ± .9	**6.7 ± .8**	**7.0 ± .9**
Gynoid Lean Mass (lbs)	19.6 ± 1.4	19.7 ± 1.3	**14.4 ± 1.4**	**14.9 ± 1.6**
Waist Circumference (cm)	76.4 ± 2.2	75.1 ± 3.7	69.6 ± 2.6	66.3 ± 2.0
Hip Circumference (cm)	93.4 ± 1.9	93.4 ± 3.0	90.8 ± 3.3	91.2 ± 3.8
Thigh Circumference (cm)	51.6 ± 2.1	51.0 ± 2.9	**49.7 ± 2.4**	**48.6 ± 2.5**

- Data are presented as mean ± SD
- Data in bold is significantly ($p < .05$) different from pre- to post

Table 5: Lumbar Spine Bone Mineral Density (BMD) Pre and Post Cross-Country Season and Normal Reference Values

	Males		**Females**		**Normal**	
	Pre- CC	Post- CC	Pre- CC	Post- CC	Males	Females
L1 BMD (g/cm^2)	1.01± .10	1.01 ± .10	**1.00 ± .08**	**.97 ± .08**	.98 ± .11[c]	.96 ± .11[c]
L2 BMD (g/cm^2)	1.15 ± .13	1.17 ± .13	1.09 ± .08	1.07 ± .09	1.08 ± .11[c]	1.07 ± .12[c]
L3 BMD (g/cm^2)	1.23 ± .15	1.22 ± .17	1.19 ± .11	1.18 ±.10	1.08 ± .12[c]	1.10 ± .11[c]
L4 BMD (g/cm^2)	1.18 ± .12	1.19 ± .12	1.15 ± .08	1.17 ± .07	1.07 ± .12[c]	1.09 ± .11[c]
L1-2 BMD (g/cm^2)	1.08 ± .11	1.09 ± .11	**1.05 ± .08**	**1.02 ± .08**	NA	NA
L1-3 BMD (g/cm^2)	1.14 ± .13	1.14 ± .13	**1.10 ± .08**	**1.08 ± .09**	NA	NA
L1-4 BMD (g/cm^2)	1.15 ± .12	1.15 ± .12	1.11 ± .08	1.10 ± .08	1.22 ± .08[b]	1.12 ± .08[b]
L2-3 BMD (g/cm^2)	1.19 ± .14	1.20 ± .15	1.14 ±.09	1.13 ± .09	NA	NA
L2-4 BMD (g/cm^2)	1.19 ± .13	1.19 ± .14	1.15 ± .08	1.14 ± .08	NA	1.24 ± .11[a]
L3-4 BMD (g/cm^2)	1.20 ± .13	1.22 ± .14	1.17 ± .09	1.17 ± .08	NA	NA

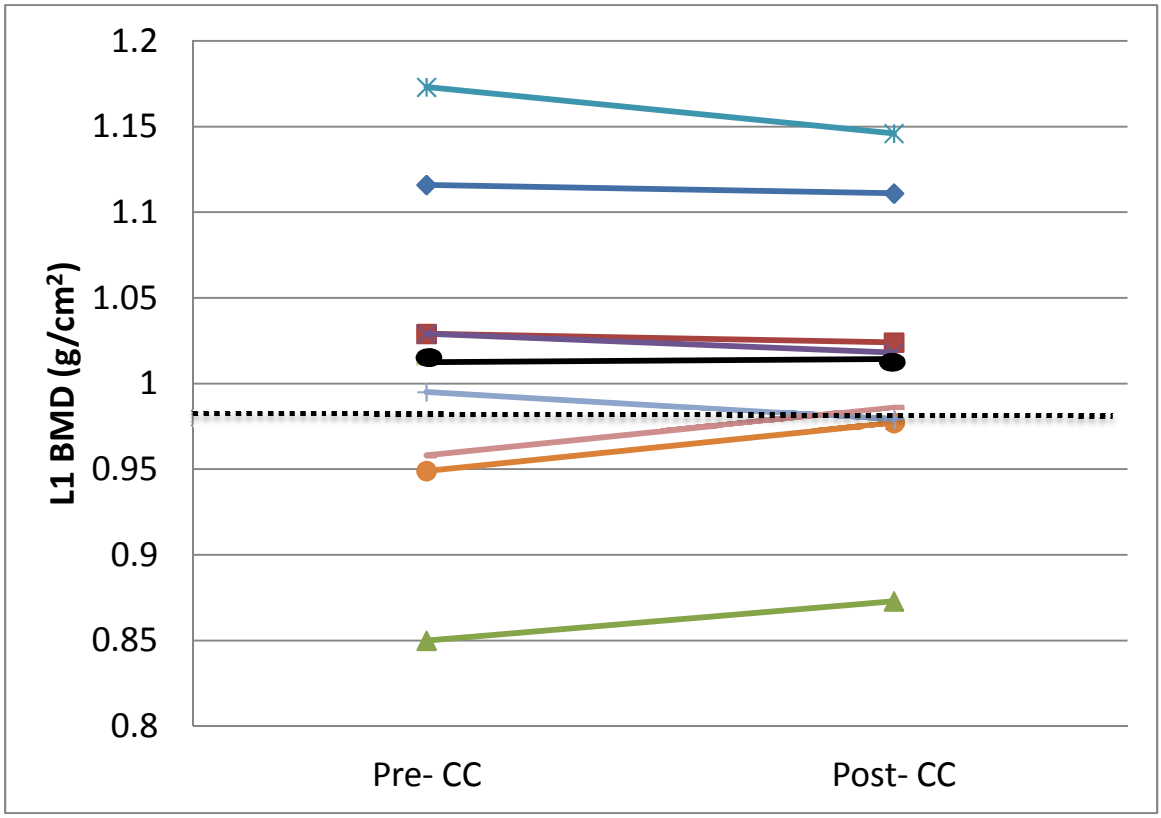
- Data are presented as mean ± SD
- Data in bold is significantly ($p < .05$) different from pre- to post
- NA = not available/applicable

(a) See reference [99]

(b) GE ilunar DXA reference values

(c) See reference [101]

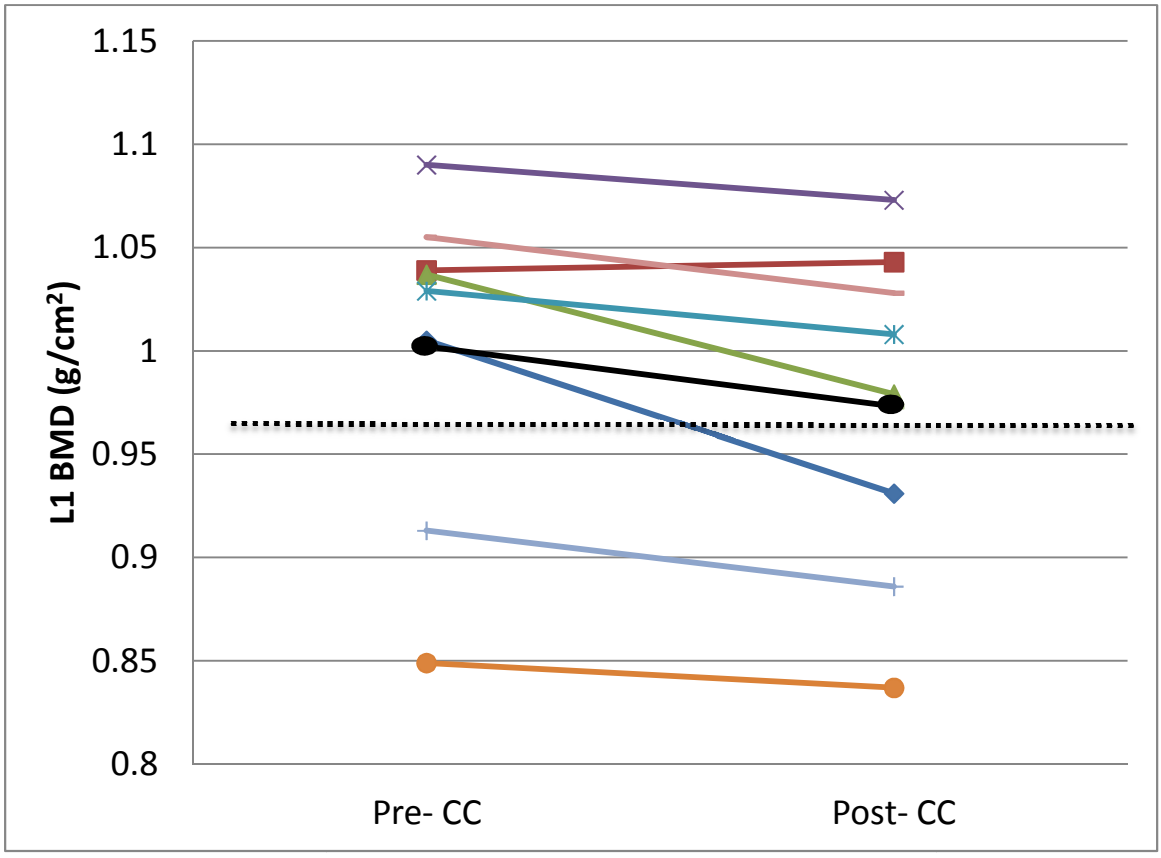
Figure 5: Bone Mineral Density of First Lumbar Spine in Individual Male Subjects Pre-Post Cross-Country Season



Represents Group Mean

Represents Reference Value (See reference [101])

Figure 6: Bone Mineral Density of First Lumbar Vertebrae in Individual Female Subjects Pre-Post Cross-Country Season



Represents Group Mean

Represents Reference Value (See reference [101])

Table 6: Forearm Bone Mineral Density (BMD) Pre and Post Cross-Country Season and Normal Reference Values

	Males (n= 8)		**Females (n= 8)**		**Normal**	
	Pre-	Post	Pre-	Post	Male	Female
Ultra-Distal Radius BMD (g/cm^2)	.45 ± .06	.44 ± .06	.38 ± .03	.33 ± .12	.50 ± .05[a]	.47 ± .05[a]
Ultra-Distal Ulna and Radius BMD (g/cm^2)	.41 ± .06	.40 ± .05	.35 ± .02	.34 ± .02	.49 ± .05[b]	.38 ± .05[b]
33 % of Radius Length BMD (g/cm^2)	.89 ± .07	.90 ± .07	.77 ± .08	.79 ± .08	1.0 ± .10[a]	.88 ± .09[a]
Radius Total BMD (g/cm^2)	.67 ± .06	.66 ± .06	.59 ± .04	.58 ± .04	.76 ± .07[a]	.68 ± .05[a]
Ulna Total BMD (g/cm^2)	.63 ± .07	.63 ± .07	.54 ± .03	.53 ± .03	NA	NA
Radius and Ulna Total BMD (g/cm^2)	.65 ± .06	.65 ± .06	.57 ± .04	.56 ± .04	NA	NA

- Data are presented as mean ± SD
- Data in bold is significantly ($p < .05$) different from pre- to post
- NA = not available/applicable

(a) GE ilunar DXA reference values

(b) See reference [102]

Hip and Femur

The average (± SD) values for hip and femur BMD pre- post CC are shown in Table 8. Male subjects did not show any significant changes in BMD of the hip and

femur regions, other than a trend to increase trochanter mean BMD (p= .06). Females did not show any significant changes in hip and femur BMD from pre- post CC.

Furthermore, hip and femur BMD measures for males and females in this study did not differ significantly from normal reference data values/ranges pre- CC or post CC.

Table 7: Hip and Femur Bone Mineral Density (BMD) Pre and Post Cross-Country Season and Normal Reference Values

	Males (n= 8)		**Females (n= 8)**		**Normal**	
	Pre-	Post	Pre-	Post	Male	Female
Femur Neck Mean BMD (g/cm^2)	1.14 ± .09	1.15 ± .09	1.06 ± .12	1.07 ± .13	.88 ± .12[a]	.79 ± .09[a]
Ward's Triangle Mean BMD (g/cm^2)	1.04 ± .12	1.04 ± .11	.96 ± .13	.97 ± .14	.79 ± .16[a]	.69 ± .11[a]
Trochanter Mean BMD (g/cm^2)	.93 ± .08	.94 ± .08	.86 ± .10	.86 ± .12	.75 ± .11[a]	.69 ± .11[a]
Femur Shaft Mean BMD (g/cm^2)	1.35 ± .12	1.37 ± .11	1.26 ± .13	1.27 ± .12	NA	NA
Total Hip/Femur Mean BMD (g/cm^2)	1.14 ± .08	1.14 ± .08	1.07 ± .11	1.07 ± .11	1.02 ± .13[a]	.89 ± .11[a]

- Data are presented as mean ± SD
- Data in bold is significantly (p< .05) different from pre- to post
- NA = not available/applicable

(a) See Reference [103]

Blood Measures

The average (± SD) values for blood measures for pre and post CC are shown in table 9. Males showed a significant decrease in vitamin D levels pre- to post CC.

Males also showed trends for a decrease in estrogen (p= .09), magnesium (p= .05), and ferritin (p= .06) pre- post CC. Females showed a significant decrease in luteinizing hormone and magnesium but an increase in iron levels pre- to post CC.

Potential Predictors of Total and Regional Bone Mineral Density

A Correlation Matrix between total and regional BMD measures and potential predictors for males and females are presented in Tables 9 and 10. Total body BMD and as well as radius and ulna total BMD showed a significant positive correlation with lean body mass (r= .79 and .83, respectively) in males. Total hip and femur BMD showed a significant inverse correlation with running mileage (r= -.79) in males. Males did not show any other significant correlations between total body, spine, hip, or forearm BMD with caloric intake, running mileage, percent body fat, lean body mass, or testosterone levels.

Total hip and femur BMD showed a significant inverse correlation with percent body fat (r= -.72) in females. Females did not show any other significant correlations between total body, spine, hip, or forearm BMD with caloric intake, running mileage, percent body fat, lean body mass, or testosterone levels.

Table 8: Blood Measures Pre and Post Cross-Country Season and Normal Reference Ranges

	Male (n= 7)		**Female (n= 7)**		**Normal**	
	Pre-	Post	Pre-	Post	Male	Female
LH (mIU/mL)	4.6 ± 1.6	5.1 ± 2.0	**12.6 ± 14.6**	**4.4 ± 3.5**	1.7 - 8.6[a]	NA
FSH (mIU/mL)	3.13 ± 1.6	3.40 ± 1.6	4.1 ± 2.6	3.5 ± 1.8	1.5 - 12.4[a]	NA
Vitamin D, 25-Hydroxy (ng/mL)	**42.03 ± 7.7**	**32.9 ± 6.9**	42.9 ± 11.7	37.4 ± 9.9	30 – 100[a]	30.0 - 100[a]
Testosterone, serum (ng/dL)	455.4 ± 118.4	438.1 ± 73.8	37.9 ± 19.5	30.9 ± 17.4	348 – 1197[a]	5 – 38[a]
Estrogen, total (pg/mL	89.1 ± 10.5	73.4 ± 17.6	178.6 ± 93.1	113.7 ± 51.3	40 – 115[a]	NA
Calcium, ionized, serum (mg/dL)	4.9 ± .2	5.0 ± .2	5.0 ± .1	5.1 ± .2	4.5 - 5.6[a]	4.5 - 5.6[a]
Magnesium, serum (mg/dL)	2.0 ± .1	2.1 ± .2	**1.93 ± .1**	**2.07 ± .1**	1.6 - 2.6[a]	1.6 - 2.6[a]
Iron, serum (ug/dL)	124.3 ± 96.5	132.1 ± 58.7	**54.6 ± 22.9**	**91.1 ± 28.4**	40 – 155[a]	35 – 155[a]
Ferritin, serum (ng/mL)	66.6 ± 19.5	53.0 ± 17.5	31.9 ± 18.1	29.6 ± 16.7	30 – 400[a]	15 – 150[a]

- Data are presented as mean ± SD
- Data in bold is significantly ($p < .05$) different from pre- to post
- NA = not available/applicable

(a) Normative data provided by LabCorp, Inc.

Table 9: Correlation Matrix of Total and Regional Bone Mineral Density Versus Potential Correlates in Male Subjects

	Total Body BMD	Spine BMD (L1)	Hip BMD (Total Hip and Femur)	Forearm BMD (Radius and Ulna Total)
Energy Intake (kcal/day)	.04	.13	.00	.18
Running Mileage (mi/wk)	-.52	-.35	**-.72**	-.34
Body Fat % (DXA)	-.40	-.19	-.56	-.32
Lean Body Mass (lbs)	**.79**	.41	.64	**.83**
Testosterone (ng/dL)	.17	-.20	-.13	.12

- Data in bold is significantly ($p < .05$) different from pre- to post
- Spearman Correlation Coefficient (r) are presented

Table 10: Correlation Matrix of Total and Regional Bone Mineral Density Versus Potential Correlates in Female Subjects

	Total Body BMD	Spine BMD (L1)	Hip BMD (Total Hip and Femur)	Forearm BMD (Radius and Ulna Total)
Energy Intake (kcal/day)	-.56	.30	.20	-.30
Mileage (mi/wk)	.33	-.45	-.03	.66
% Body Fat (DXA)	-.37	-.64	**-.72**	-.19
Lean Body Mass (lbs)	.31	.69	.14	-.26
Testosterone (ng/dL)	.07	-.21	-.25	.14

- Data in bold is significantly ($p < .05$) different from pre- to post
- Spearman Correlation Coefficient (r) are presented

DISCUSSION

Several studies have evaluated bone mineral density (BMD) in athletes[27,40,62,98–100]. The majorities of these studies have been cross-sectional and have only included females[40,104–107]. The limited studies on male athletes have produced inconclusive and conflicting results and do not yet provide a definitive conclusion as to the effects of endurance running on bone mineral density[44,108,109]. Therefore, it is the aim of this investigation to identify the changes in BMD in both female and male athletes over the duration of a competitive Cross-Country season of approximately three months. Furthermore, this investigation sought to identify the factors that may influence BMD in both female and male Cross-Country runners.

Participant Demographics

The participants of the study were similar in age, body mass index, body composition, running mileage, and fitness level as those in other studies[44,104,109,110]. At pre- Cross-Country season (pre- CC) the runners in this study were at a high level of fitness, indicated by the reported running mileage and VO_2 maximum of 71.8 and 59.9 ml/kg/min in males and females, respectively. These athletes also had a low body mass index (BMI) at pre- CC because of they were already training at a high level. Consequently their pre- CC training may have potentially reduced the change observe in this study measured over the Cross-Country season.

Dietary Analysis Results

Only 12 subjects completed the dietary log necessary to analyze nutrient intake. More males (n= 7) then females (n= 5) completed the dietary log. Thus, the collected data may not represent the groups accurately enough to make any definitive conclusions based on dietary intake. As expected, that males consumed more calories than females. Based on running mileage, males and females did not consume sufficient calories to meet their energy needs[97]. Males also consumed significantly more cholesterol compared to females. This may be the result of one female athlete being vegetarian and not consuming cholesterol. Additionally, based on the diet logs males ate higher amounts of red meat than females. Neither group had intakes of nutrients that were significantly different than reference values/ranges. This may be miss-leading due to the difficulty of identifying intake values for athletes that engage in high levels of daily physical activity. Dietary guidelines are used for the general public and do not apply to endurance athletes. Therefore, individual dietary recommendations are required for each athlete based on energy expenditure to accurately assess adequate nutrition intake.

Changes in Body Composition Pre-Post CC

As expected males had higher total body mass, lean mass, total BMD, and total bone mineral content than females at pre- CC. In contrast, females had a higher body fat percentage and fat mass than males at pre- CC. While there were no significant changes in total body mass from pre- post CC in males or females, females did show a significant increase of 2.7 (±1.7) lbs in total lean mass. Although not significant, males also showed

an increase of 2.1 (± 3.8) lbs in total lean mass. The increase in lean body mass in both males and females could be due to the increase of muscle mass as bone mass did not change during the study. This represents an increase of 2.1 % and 1.4 % of total mass in females and males, respectively. The increase in muscle mass may be due to the resistance training that was adopted during the competitive season.

While there were no significant changes in BMD from pre- post CC in males or females, the findings of this study still provide some reason for concern. Of the eight males and eight females evaluated for change in BMD (Figures 3 and 4), four males and four females were at or below the "normal" level at pre- CC and during the course of this study. While a competitive Cross-Country season did not appear to decrease BMD, the 50% of athletes with low BMD levels are at risk for stress fractures and osteoporosis later in life.

Additionally, females showed significant increases in trunk, android, and gynoid lean mass which is the probable cause of the significant increase in total body lean mass. This increase in lean mass may be due to exercises performed by the runners that were not related to running, such as resistance training. Cross-Country runners at WFU performed core strengthening exercises 2-3 times per week during the season. Females may have showed a greater increase in muscle mass since they started with a lower pre-CC level than males.

Changes in Regional Bone Mineral Density Pre- Post CC

Males did not show any significant changes in lumbar spine BMD from pre- to post CC whereas females demonstrated significant decreases in L1, L1-2, and L1-3 BMD. This represents a reduction of 2.9 %, 2.2 %, and 1.6 % g/cm^2 in these regions of their spine for females. This finding is consistent with Hind et al.,[77] who identified that trained female endurance runners had a reduced lumbar spine BMD when compared to a reference population. The current study is the first to show these changes over the course of one season. Although this change may seem slight, and additive change over numerous competitive seasons may lead to clinically significant reductions in lumbar spine BMD. A decrease in lumbar spine BMD is of particular concern as this is a common site of osteoporotic fractures in older females[111].

Males did not show any significant changes in forearm BMD levels and females only showed a trend (p=.06) for a decrease in radius total BMD. A larger number of subjects would have likely allowed this to reach statistical significance. In a cross sectional study Heinonen et al.,[106] determined that female endurance athletes had lower forearm BMD levels compared to female athletes of other sports. While not strongly supported by the findings of the current study, available evidence suggest that endurance activities may cause a reduction in forearm BMD levels in female endurance athletes. The mechanism for this reduction has not been well studied, but, it is believed that the lack of weight bearing exercises in the upper limbs in combination with low estrogen levels may be responsible for the reduction in forearm BMD[112].

Females did not show a significant change in BMD measures of the hip and femur, while males trended to increase trochanter mean BMD (p= .06). It has been shown that

the “loading” associated with moderate amounts of running and other weight bearing endurance exercises can increase BMD levels[27]. However, higher levels of running have been shown to decrease BMD. The lack of change in hip and femur BMD may be due to the moderate volume/mileage of training of these athletes during the season and/or the relatively short duration (3 months) of the study.

Change in Blood Measures Pre- Post CC

Males in this study showed a significant decrease in Vitamin D levels pre- to post CC, which has been observed in a previous study of endurance athletes[113]. While the results of this study support the evidence that endurance running can lower Vitamin D levels in athletes, it is not well understood as to why this occurs. Some evidence suggests that an inflammatory response may effect Vitamin D levels. This decrease in vitamin D levels is of concern as it has been associated with a higher incidence heart disease and cancer[114,115]. Males also showed trends for a decrease in estrogen (p= .09), magnesium (p= .05), and ferritin (p= .06) pre- post CC. A reduction in ferritin levels in male endurance athletes has been observed in other studies[116,117]. It is believed that endurance training can cause an increase in iron loss through sweet and a reduction in iron absorption resulting in the depletion of ferritin stores over time[118]. Low ferritin levels have been shown to reduce athletic performance in endurance athletes[119]. Magnesium reductions are seen after acute bouts of intense aerobic exercise[120]. This occurs through mineral loss from sweat and muscle uptake of magnesium during exercise. There is no evidence that endurance activity causes chronic reductions in serum magnesium.

Therefore, the reduction in serum magnesium in this study may be caused by training or racing the day prior to testing.

Females showed a significant decrease in luteinizing hormone and magnesium but an increase in iron pre- to post CC. Luteinizing hormone, magnesium, and iron levels change with menstrual status in females[121,122]. We did not control for menstrual status in this study, thus, it is difficult to draw conclusions about these changes. The increase in iron was probably due to a change in iron supplementation during the season. At pre- CC, one athlete started taking iron supplementation as she was severely anemic. Based on questionnaire data, several females indicated they had been anemic in the past and now routinely take iron supplements.

Potential Correlates of Total and Regional Bone Mineral Density

Potential predictors of BMD of the total body, L1, hip and femur and forearm were examined in male and female runners. Due to the small homogenous nature of this sample, it was somewhat surprising to find that there were any significant correlates of BMD levels in the athletes. Despite this, at pre- CC males showed significant correlations between total BMD, as well as forearm BMD, with total lean body mass. Previous studies have indicated that total body lean mass is generally predictive of BMD in males[37]. In the present study hip BMD in males was negatively correlated with running mileage at pre-CC. This was unexpected and inconsistent with the literature as most studies have shown that BMD increases in the lower limbs from weight bearing exercise, including running[13,25,40]. However, MacDougall[44] found that BMD tended to decrease after a

threshold of greater than 30 miles per week. The relationship of mileage to BMD may be due to the inability of the bone to recover as efficiently from repetitive damage and result in a reduced BMD. Male runners in this study averaged 70 miles per week at the beginning of the season and maintained a high amount of mileage throughout the study.

While there was no significant relationship between total BMD and percent body fat, females in this study had a negative correlation between hip BMD and percent body fat pre- CC. Hage et al[37]., found that fat mass was a strong predictor of total BMD but, Weiler et al[123]., found that fat mass was inversely correlated with total BMD. Neither of these two studies investigated the relationship between fat mass and BMD of the hip.

Study Limitations and Future Research

Although this study is the first study to evaluate the BMD levels in male and female runners from pre- post CC, this study is not without limitations. This study was limited by the small sample size and the homogeneity of the subjects. While a sample of white non-Hispanic, highly trained Cross-Country runners may not represent the majority of endurance athletes, runners of other ethnicities and fitness levels need to be included before making broad generalizations on BMD in Cross-Country runners. Another limitation of this study is the short duration of the observation period. A three month study may not be long enough to see meaningful changes in bone tissue. Furthermore, the self-reported data obtained for some measures in this study also reduces the reliability of the purposed findings.

Further studies need to be conducted and include a more heterogeneous mix of competitive distance runners and to follow them over a longer duration. Ideally, athletes could be tested at entry to college and throughout their competitive years and even for decades after college to fully evaluate the effects of distance running on bone health.

CONCLUSIONS

Despite limitations, this study adds substantially to our understanding of BMD levels of competitive collegiate Cross-Country runners. The results of this study suggest that a competitive cross country season has minimal negative or positive impact of BMD on collegiate athletes. However, approximately 50 % of the male and female athletes studied have BMD levels below their age-matched normal levels. The low BMD levels seen in these distance runners may increase their risk of stress fractures and/or osteoporosis later in life. Further investigation of this important topic is warranted.

APPENDIX A

Nutrition History Questionnaire

ID. _____ Date_____

Weight History:
Childhood ___Underweight ___ Normal ___ Overweight ___ Obese
Teenage ___Underweight ___ Normal ___ Overweight ___ Obese
20‘s ___Underweight ___ Normal ___ Overweight ___ Obese

Do you follow a specific diet? (ex. Vegan, vegetarian, low carbohydrate, etc.)
( ) Yes ( ) No

If Yes, Specify:_____

Do you have any food allergies/Intolerances? ( ) Yes ( ) No
If Yes, Specify:_____

Do you take any vitamin, mineral or food supplements? ( ) Yes ( ) No
If Yes, List_____

_____

Have you been advised by your physician to follow a type of diet? ( ) Yes ( ) No
If Yes, Type of Diet: ___ No Salt ___ Low Cholesterol ___ No Sugar _____Other

******* The following are questions about your typical eating patterns *******

How many days per week do you eat? (Breakfast)_____ (Lunch)_____(Dinner)_____

How often do you snack? ( ) once daily ( ) twice daily ( ) three or more times daily

When do you usually snack? ( ) mid-morning ( ) mid-day ( ) after dinner

Do you eat out? ( ) Yes ( ) No If Yes, How often?_____

Type of restaurants?_____

Do you drink alcohol? ( ) Yes ( ) No # of drinks/week ____

APPENDIX B

Athlete 7-Day Food Log

Name:_____ **Date:**

from _____ to _____

Day	Food Item(s)	Serving Size (cups, oz. etc)	# of Servings	# of Calories (if know

APPENXID C

Running History Questionnaire

ID. _____

1.) How long have you been running competitively? (years or months)

_____

2.) Please list your Personal Best Times (4 events)

Distance and Time

1_____

2_____

3_____

4_____

3.) Have you run any races in the past 3 months?

No _____
Yes _____

*If you checked "Yes" please fill in your finish time(s) on whatever distance(s) you ran:

5K _____

10K _____

Other Distance (Please specify) _____

4.) How many miles per week did you run this past week?

_____miles

5.) How many miles per week on average did you run for the past month?

_____miles

6.) How many miles per week on average did you run for the past 3 months?

_____miles

7.) Describe your typical week training for the past month. (diagram)

- Include duration (how long was the run/workout), pace (min/mile or interval), and distance (miles run for whole run)

Mon.	Tues.	Wedn.	Thurs.	Fri.	Sat.	Sun.

8.) What surfaces do you typically train on? (Eg. Grass, asphalt, concrete sidewalks, track surface)

_____

10.) What type of shoe have you been wearing for the past month? (make and model)

_____

11.) Have you had a stress fracture in the past?

No _____

Yes _____

If yes, when did it occur and in what bone

_____

Was this a physician diagnosed stress fracture?

No_____

Yes_____

APPENDIX D

Running Log

Week # ____

Daily run/workout	Time/Dist.	Other Activities	Daily total
Mon.			
Tues.			
Wedn.			
Thurs.			
Fri.			
Sat.			
Sun.			

APPENDIX E

Female Athlete Questionnaire

ID. _____

1.) Have you started your menstrual cycle?

Yes_____

No_____

- If yes, at what age?

_____years old

2.) In the past month have you had your menstrual cycle?

Yes_____

No_____

3.) In the past 6 months how many times have you had your menstrual cycle?

_____times

4.) In the past year, how many times have you had your menstrual cycle?

_____times

5.) What is the longest time between menstrual periods in the last year?

_____months

6.) Do you ever miss periods or have changes in the frequency of your menstrual cycle?

Yes_____

No_____

7.) Have you taken birth control medication in the last year?

Yes_____

No_____

8.) Are you currently using birth control medication

Yes_____

No_____

9.) How long have you been taking birth control medication?

_____(years and/or months)

10.) Are you on any other medication that affects your menstrual period?

Yes_____

No_____

- If yes what medication

_____

APPENDIX F

Follow-up Questionnaire

ID. _____

1.) Have you run any races this season?
Yes _____

No _____

*If you checked "Yes" please fill in your finish time(s) on whatever distance(s) you ran:

5K _____

6K_____.

8K _____.

10K _____

Other Distance (Please specify) _____

How many miles per week did you run this past week?

_____miles

How many miles per week on average did you run for the past month?

_____miles

How many miles per week on average did you run for the past 3 months?

_____miles

2.) Describe your typical week training for the past month. (diagram)

- Include duration (how long was the run/workout), pace (min/mile or interval), and distance (miles run for whole run)

Mon.	Tues.	Wedn.	Thurs.	Fri.	Sat.	Sun.

3.) What type of shoe have you been wearing for the past month? (make and model)

_____

4.) Have you had any injuries that have prevented you from training or racing this season?

No _____

Yes _____

If yes, please describe.

_____

When is the last date of training / competition? ._____.

5.) Do you take any vitamin, mineral or food supplements? ( ) Yes ( ) No

If Yes, List_____

Did this change during the season? ( ) Yes ( ) No

If yes,
how _____

6.) Do you drink alcohol? ( ) Yes ( ) No # of drinks/week ____

7.) Do you drink caffeinated beverages (sodas, coffee, etc)? ( ) Yes ( ) No

# of drinks/week

Females Only

8.) In the past month have you had your menstrual cycle?

Yes_____ If yes, what was the date of your last menstruation
ovulation _____

No_____

9.) In the past 3 months how many times have you had your menstrual cycle?

_____times

10.) Are you currently using birth control medication

Yes_____

No_____

11.) If yes, how long have you been taking birth control medication?

_____(years and/or months)

12.) Are you on any other medication that affects your menstrual period?

Yes_____

No_____

If yes, what medication

_____

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CURRICULUM VITAE

Education

2008 - 2012 | Bachelor of Science Degree in Exercise Science

- Studied movement and exercise science at Purdue University, IN (2008-2009)
- Degree in exercise science from Gannon University, PA (2009-2012)
- Cumulative G.P.A of 3.55
- Dean's List for fall (2010) and spring (2011) semesters
- Cum laude graduate
- Demonstrating high levels of knowledge in kinesiology, anatomy, and physiology
    - Class work pertaining to research methodology, statistics, exercise physiology, exercise prescription, and motor learning
    - Lab experience includes exercise physiology, cadaver based anatomy, and animal physiology

2012 - 2014 | Master of Science Degree in Health and Exercise Science

- Currently attending Wake Forest University
- Cumulative G.P.A of 3.40
- Thesis based graduate program
- Graduate level course work in the areas of physiology, epidemiology, research design, statistics, psychology, biomechanics, and cardiopulmonary disease
    - Experience working in clinical settings performing ECG stress testing, blood pressure measurements, and exercise prescription
    - Exposure to large randomized clinical trials, grant and thesis writing, and teaching
    - Familiarity in working with older adults and disease populations

Job Experience

08-2012 to current | Wake Forest University Instructor

- Teaching Health and Exercise (HES 101)
- Presenting lectures and conducting labs
- Lecturing on physiology, biomechanics, exercise prescription, and nutrition

08-2012 to current | HELPS Student Staff

- Over 400 hours of clinical experience
- Performing ECG stress tests
- Leading stretching and resistance programs
- Conduction body composition assessments

4-2013 to current | HES 354 Lab Assistant

- Calibration of equipment
- Operation of metabolic measurement instruments
- Setting-up lab materials

5-2008 to 8-2012 | Landscaper

- Coordination of employees at job sites
- Managing money for supplies
- Maintaining and operating machinery and equipment

Volunteer Work

3-2010 to 5-2010 | Volunteer High School/Elementary Coach

- Conditioning of young athletes in endurance events
- Monitoring workouts and teaching skills
- Operating timing system during meets

4-2008 to current | Volunteer for The Village at Luther Square

- Aid in activities for elderly residents
- Help staff transport residents on outings
- Supported community and cultural events

6-2012 | Benefit Race Organizer

- Held a fund raiser for the North East High School track program
- Involved organizing a summer track meet
- Raised over $200 from donations and participation

Extracurricular/Awards

- Science Research Fund Co-recipient (Wake Forest University)
- Varsity track at Purdue University, West Lafayette IN
- Varsity cross country at Gannon University, Erie PA
- NCAA All-Academic cross country 2010
- 30+ hours of Osteopathic physician shadowing