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Effects of Omega-3 polyunsaturated fatty acids on bone mineral density and overuse injuries in collegiate female athletes

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Effects of Omega-3 Polyunsaturated Fatty Acids on

Bone Mineral Density and Overuse Injuries in Collegiate Female Athletes

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Abstract

Lower limb overuse injuries are prevalent in female athletes and can lead to significant time lost in participation. Overuse injuries such as stress fractures occur with repeated microdamage to bone, ligaments and tendons. Numerous geometric features of bone, including bone mineral density (BMD), have been associated with stress fracture risk. While Omega-3 polyunsaturated fatty acids (PUFAs) have been associated with reducing inflammation, increasing BMD, and reducing incidence of osteoporotic fractures in the elderly, no studies have addressed the role of Omega-3 PUFAs in reduction of injury risk in young individuals. Studies indicate that Omega-3 PUFAs act on cytokines to inhibit osteoclasts while also promoting osteoblasts, thereby preventing bone resorption and stimulating bone formation. We examined the hypothesis that athletes with higher circulating and dietary levels of Omega-3 PUFAs will have higher BMD and a lower history of overuse injuries, including stress fractures, than athletes with lower levels of Omega-3 PUFAs. We distributed a food frequency questionnaire (FFQ) directed at collecting Omega-3 PUFAs and used dual-energy x-ray absorptiometry to measure phalangeal BMD in 125 athletes. Circulatory Omega-3 PUFAs were quantified in red blood cells of 23 athletes. Athletes with a history of overuse injuries had significantly lower percentages of circulatory Omega-3 PUFAs ($p = 4.33 \times 10^{-2}$) and significantly lower BMD ($p = 2.0 \times 10^{-2}$) than athletes with no history of overuse injury. However, we found no relationship between BMD and circulatory Omega-3 PUFAs ($r^2 = 6.2 \times 10^{-3}$) and were unable to reproduce circulating levels of fatty acids with our FFQ. While these data suggest Omega-3 PUFAs may influence bone health and injury risk in young athletes, especially those engaged in sports involving repetitive loading, the factors influencing bone health in young athletes are complex. Understanding the relationships among dietary factors, musculoskeletal health and injury risk is essential to the development of prevention strategies for stress fractures and other musculoskeletal injuries.

Introduction

Considerable research has been devoted to identifying risk factors for stress fractures in an effort to minimize the occurrence of injury in young athletes and military recruits, however, few studies have addressed the role of nutrition, especially fatty acids, in the reduction of injury risk. We will examine the relationships among Omega-3 polyunsaturated fatty acids (PUFAs), bone mineral density (BMD), and overuse injury risk in female athletes.

Injuries can be acute or overuse. Overuse injuries occur over time due to wear and tear on the body, most notably during repetitive loading. The most common lower limb overuse injuries in female athletes include knee pain, stress fractures (microtears in bone) and patellar tendonitis (Almeida, 1999; Borowski, 2008; Iwamoto, 2008). Acute injuries occur due to a single event, such as a twist, fall, or blow to the knee, in which those highest in frequency include ACL tears, ankle sprains (stretched ligament), meniscus injuries, and strains (pulled muscle; Almeida, 1999; Borowski, 2008; Iwamoto, 2008). In a study of a Collegiate Athletic Association Division I university over a period of three years conducted by Yang *et al.*, 2012, overuse injuries were more prevalent in females than males, in which the highest rates were found among female field hockey, soccer, softball and volleyball teams. Compared to acute injuries, overuse injuries tended to be more severe and approximately half of all overuse injuries led to time lost in participation; in particular, 82.4% of stress fractures contributed to time lost in participation.

There are several types of fatty acids, including saturated fatty acids (SFAs; hydrocarbon tail contains all single bonds), monounsaturated fatty acids (MUFAs; hydrocarbon tail contains one double bond), PUFAs (hydrocarbon tail contains at least two double bonds in cis conformation), and trans-fatty acids (PUFAs which are naturally in or have been hydrogenated to the trans conformation). There are two major types of PUFAs, Omega-6 and Omega-3, which are known as essential fatty acids (EFAs) because they cannot be synthesized by the body and must be acquired through diet. Omega-6 PUFAs, such as linoleic acid (LA) and arachidonic acid (AA), and Omega-3 PUFAs, such as α-linolenic acid (ALA) and parinaric acid, can be acquired from liquid vegetable oils, nuts, seeds, butter, meat, poultry, eggs, or fish, in which Omega-3 PUFAs are present in smaller quantities. The Institute of Medicine recommends 1.1 g/day of dietary Omega-3 PUFAs for females, as deficiencies have been associated with decreased cognitive function (Fontani and Migliorini, 2012), which may affect athletic performance. Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docasahexaenoic acid (DHA), are also Omega-3 PUFAs which are mostly acquired from fatty fish and have been widely associated with benefits such as reducing inflammation (Simopoulous, 2007), mitigating atherosclerosis and coronary heart disease (León, *et al*., 2008; Von Schacky *et al.*, 1999) and reducing the risk or severity of osteoporosis (Moon *et al*., 2012). It is important to acquire EPA and DHA from dietary sources of fatty fish, like salmon and albacore tuna, because the human body cannot convert ALA from diet into EPA and DHA in sufficient amounts (Kruger, 2010; Salari, 2008).

Several fatty acids have been studied for their influence on bone health, particularly on their role in calcium metabolism, but PUFAs have been most notably examined for their wide array of health benefits and their role on bone health for the purposes of reducing osteoporotic fracture risk. For example, SFAs have been negatively associated with BMD (Corwin *et al.*, 2006; Kruger *et al.*, 1998) and have been correlated with decreasing intestinal absorption of calcium (Ateh and Leeson, 1984; Coetzer *et al.*, 1994). Dietary MUFAs may (Martínez-Ramírez *et al*., 2007; Trichopoulou *et al*., 1997) or may not (Macdonald *et al.*, 2004) be positively correlated to bone health, depending on the source of MUFAs (e.g. plant products such as olive oil and vegetable oils versus animal products such as dairy or meat). MUFAs from a Mediterranean-like diet that is high plant sources (especially olive oil) have been shown to increase bone health and decrease fracture risk (Martínez-Ramírez *et al*., 2007; Trichopoulou *et al.,* 1997). Olive oil may be beneficial to bone health not only because contains anti-inflammatory properties (Serrano-Martinez *et al.*, 2005) that exert positive effects on BMD (Puel *et al.*, 2006), but also because it contains the MUFA oleic acid, which has been found to increase calcium absorption (Ateh and Leeson, 1984). Although one study found that MUFAs were negatively associated with BMD (MacDonald *et al.,* 2004), it is unclear if results were influenced by confounding variables. It is hard to determine the relationships between MUFAs and bone health due to limited research. Additionally, no studies similar to those conducted by Martínez-Ramirez *et al*. (2007) and Trichopoulou *et al.* (1997) have been performed in populations in which the majority of MUFAs may be from animal sources rather than from plant sources, such as in the American diet. Increased Omega-3 PUFAs have been positively correlated with factors important to bone health, like increasing intestinal calcium absorption (Coetzer *et al.*, 1994) while decreasing its excretion (Sun *et al.,* 2004; Tulloch *et al*., 1994) and thereby increasing bone strength. Studies show that increased dietary Omega-3 PUFAs (Järvinen, *et al.,* 2012; Nawata *et al,*. 2013; Rousseau *et al*., 2009; Terano, 2001) and Omega-3/Omega-6 PUFA ratios may positively affect BMD (Weiss, 2005). Increasing BMD through diet may reduce the incidence of osteoporotic fractures in the elderly.

Whether an osteoporotic or an overuse fracture, the common feature is microfractures that lead to a major injury. While an osteoporotic fracture occurs in cases of extremely decreased BMD, in addition to an event surpassing the maximum tolerable force, such as a fall, stress fractures occur due to repetitive microdamage that leads to the formation of large cracks in bone. Studies have correlated BMD with osteoporotic fractures in the elderly (Blake *et al*., 2005; Jones and Davie, 1998; Fordham *et al*., 2000; Miller *et al*., 2002) and also with overuse injuries, particularly stress fractures, in female athletes (Bennell *et al*., 1996; Lauder *et al*., 2000; Myburgh *et al*., 1990; Schnackenburg *et al*., 2011) and military recruits (Beck *et al*., 2000; Välimäki *et al*., 2005). Additional osteoporotic fracture risk factors include female gender, Caucasian race, increased age, menstrual changes (specifically decreased age at menopause), decreased calcium and vitamin D intake, high alcohol intake, history of smoking, decreased physical activity, and family history of osteopenia or osteoporosis (Chevalley, *et al.,* 1994; Dawson-Hughes *et al.,* 1990; Seeman and Allen, 1989). A study of 3758 female recruits by Lappe *et al*. (2001) discovered that many of the risk factors for osteoporotic fractures may also be risk factors for stress fractures in females, such as increased age, menstrual irregularities (specifically amenorrhea or late menarche age), decreased calcium and vitamin D intake, history of smoking, and decreased physical activity. Understanding risk factors of osteoporotic fractures has helped define many variables that may also be related to stress fracture and overuse injury risk in a younger population, however, we lack a clear understanding of predicting overuse injury risk in female athletes, rendering it necessary to evaluate additional mechanisms that may contribute to injuries in young populations.

Microdamage caused by repetitive loading induces bone remodeling, which reshapes and repairs damaged bone to prevent major damage such as injury (Martin, 1998). Compared to inactive controls, female athletes in most weight-bearing sports, e.g. volleyball and track, have increased lower limb BMD (Bloomfield, *et al*., 1993; Fehling *et al*., 1995; Heinonen *et al*., 1996; Snow-Harter *et al*., 1992; Taaffe *et al*., 1995) or accompanied increased lumbar spinal BMD (Bloomfield, *et al*., 1993; Heinonen *et al*., 1996; Snow-Harter *et al*., 1992; Taaffe *et al*., 1995).

However, repetitive microdamage to bone during repetitive loading in weight-bearing sports can also lead to stress fractures resulting from overloading and decreased BMD (Schnackenburg *et al.*, 2011). Studies have found lower BMD in cross country or long distance runners compared to other load-bearing sports (Mudd, 2007) and that stress fracture prevalence is highest among those who participate in cross country running (Field *et al.*, 2011). Continual repetitive loading in weight-bearing exercise, specifically in female runners and military athletes, may reduce bone-related benefits of exercise compared to other weight-bearing sports where overloading may not be a problem.

Remodeling occurs through the combined mechanisms of osteoclasts and osteoblasts through activation, bone resorption, and bone formation (Martin, 1998). While osteoclasts break down bone during bone resorption, osteoblasts build bone during both growth and repair (Martin, 1998). Omega-3 PUFAs increase BMD by inhibiting osteoclasts (Sun *et al.*, 2003) while also promoting osteoblasts (Bhattacharya, 2005), which produce bone and osteoprotegerin (OPG). OPG is a soluble cytokine that competes with receptor activator of nuclear factor kappa-B ligand (RANKL; Bhattacharya *et al.*, 2005; Simonet *et al.*, 1997), a surface molecule of osteoblasts, for regulation of receptor activator of nuclear factor kappa-B (RANK), a surface molecule of osteoclasts. When bound to RANK, OPG inhibits osteoclastic activity while RANKL initiates osteoclastic activity and bone resorption; increased RANKL has been correlated with decreased BMD (Sun *et al.*, 2003; Bhattacharya *et al.*, 2005). Omega-3 PUFAs may upregulate OPG (Byattacharya, *et al.*, 2005) and also reduce secretion of TNF-α from osteoclastic precursors (Baek and Park, 2013; Skuladottir, *et al.*, 2007; Sun *et al.*, 2004). TNF-α is an inflammatory factor which promotes osteoclastic activity through synergy with RANKL (Lam *et al.*, 2000). Through these mechanisms, bone resorption may be decreased during bone remodeling in the presence of Omega-3 PUFAs, which may be helpful for injury prevention, especially in cases of repetitive loading.

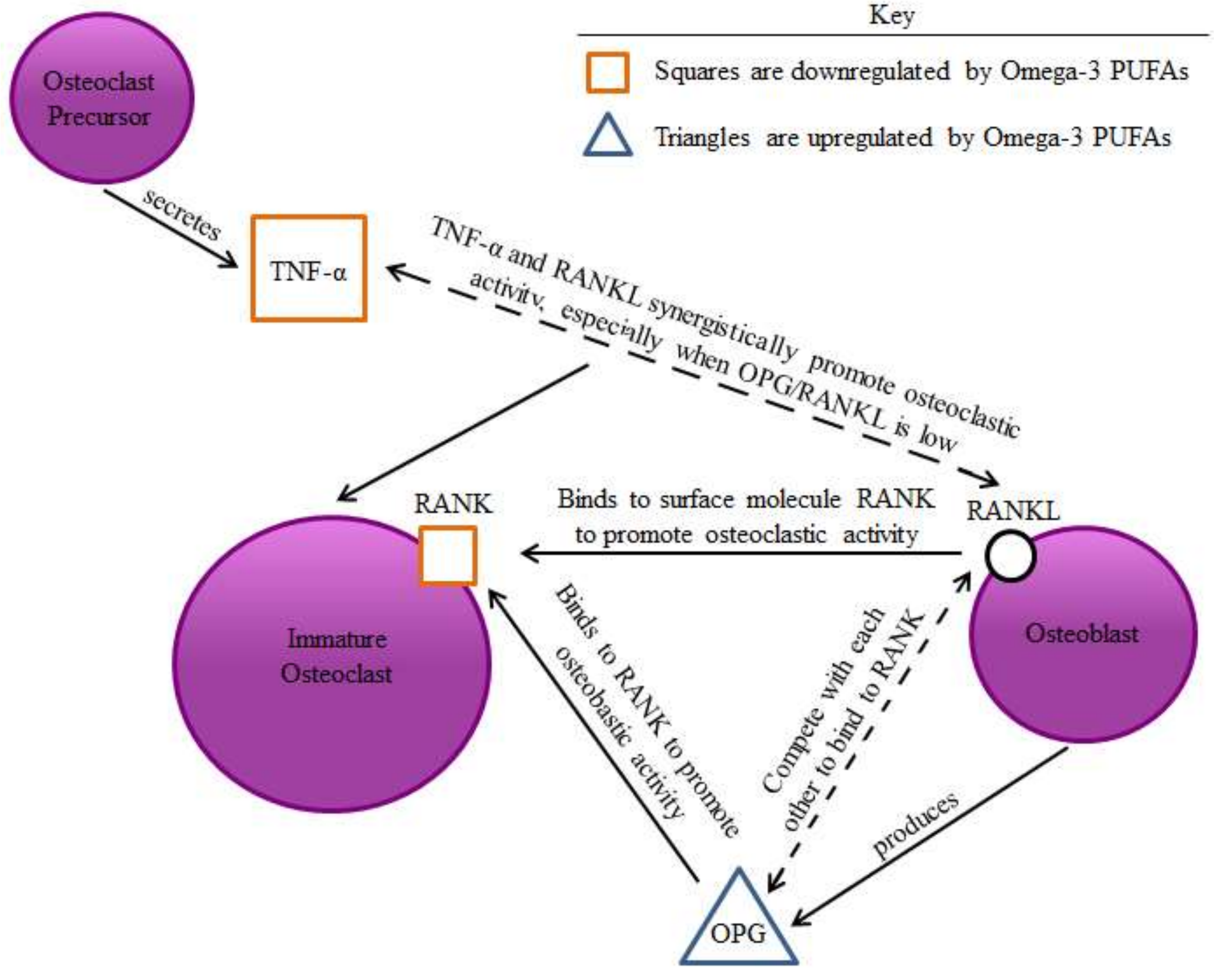


Figure 1. Overview of mechanisms involved in regulating osteoblastic activity and osteoclastic activity of bone remodeling. When bound to RANKL, RANK promotes osteoclastic activity, where TNF-α works in synergy with RANKL. When bound to OPG, RANK promotes osteoblastic activity.

There is limited research addressing the relationship among fatty acids, BMD, and stress fractures in young athletes and military recruits where the incidence of such injuries is particularly high, especially among women (Iwamoto, 2003; Macleod, 1999). Many factors have been associated with bone health and injury risk in female athletes, including hormonal fluctuations or menstrual irregularities (Bennell *et al.*, 1997; Lappe *et al.*,2001; Nattiv *et al.*, 2000) and including diet, which may be a factor that could be controlled by an athlete. Studies suggest positive influences of vitamin D and calcium intake on BMD and injury reduction (Lappe *et al.*, 2001), however, there are only a few studies that examine the role of Omega-3 PUFAs on BMD in a younger and athletic population. Damsgaard *et al.* (2012) found that fish oil supplementation of six weeks had no significant impact on BMD in adolescent boys and Eriksson, Mellström, and Strandvik (2009) found only a weak, but significant, negative association between ALA and BMD. However, Hogström, Nordström P, and Nordström A (2007) found that Omega-3 PUFA intake was positively associated with BMD in healthy men, ages 22-24 years. None of these studies assessed Omega-3 status in females athletes and the minimal and conflicting data available on Omega-3 PUFAs and BMD in a younger population represents a gap in the literature.

We seek to determine whether increased Omega-3 PUFAs positively affect BMD in young female athletes and subsequently reduce the risk of overuse injuries. We hypothesize that female athletes who have high dietary and circulating Omega-3 PUFAs will have higher BMD and a lower history of overuse injuries, including stress fractures, compared to female athletes with low circulating and dietary Omega-3 PUFAs. **We predict the following (Figure 2):**

1. Female athletes with increased dietary Omega-3 PUFAs will have increased circulatory Omega-3 PUFA levels.
2. Female athletes with increased dietary or circulatory Omega-3 PUFAs will have increased BMD.
3. Female athletes with increased BMD will be less likely to have had an overuse injury.
4. Female athletes with increased dietary Omega-3 PUFAs will have decreased incidence of overuse injury.

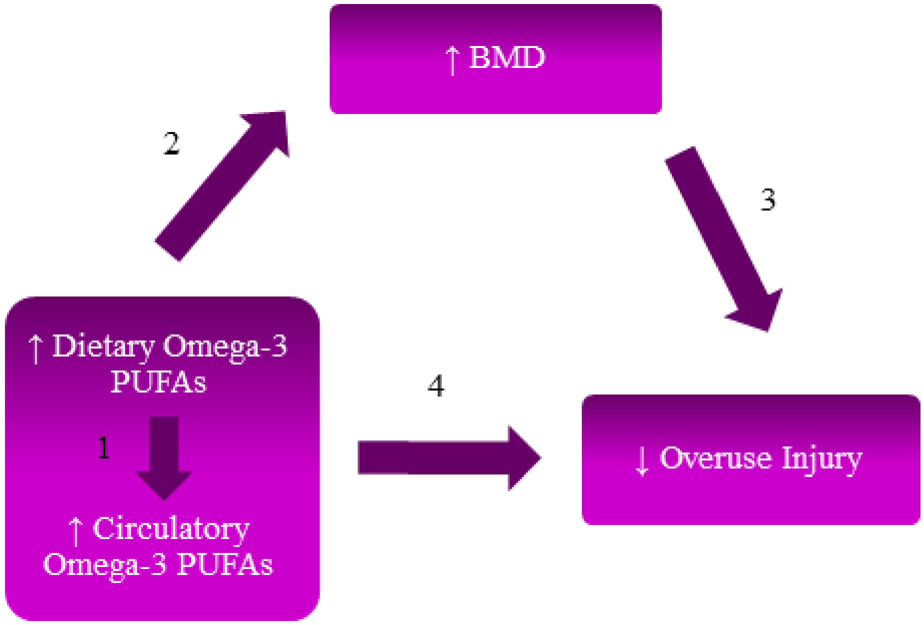


Figure 2. Overview of predictions; numbers correlate with the four predictions previously listed. We expect that females with increased dietary Omega-3 PUFAs would have increased circulatory Omega-3 PUFAs. We also predict that females with increased Omega-3 PUFAs will have increased BMD as well as decreased incidence of overuse injury.

Methods

Subjects

We selected females who were a part of an organized sport at James Madison University to participate in this study. Subjects consisted of 125 females, ages 18-22 (19.4 ± 1.2 years), from the women's basketball (n = 10), golf (n = 9), track and field (n = 23), cross country (n = 21), lacrosse (n = 25), volleyball (n = 9), softball (n = 21), club rugby (n=6), and tennis (n = 1) teams. All participants gave written informed consent and answered questionnaires on history of injuries, deficiencies, menstrual irregularities and dietary intake. We collected BMD measurements on all subjects and drew blood for fatty acid analysis in 23 subjects. This study was approved by the Institutional Review Board for Human Research at James Madison University.

At the time of consent, JMU athletic trainers and athletic training students measured height in inches and weight in pounds, without shoes. We calculated body mass index (BMI; 23 ± 3.3 kg/m^2) by dividing body weight (kg) by body height (m) squared (Table 1).

Table 1. Summary of sport categories defined for this study in addition to anthropometric data, shown as mean ± one standard deviation.

Sport Category	Sports Included	Height (m)	Weight (kg)	BMI (kg/m^2)
Cross country running sports	Cross country	1.7 ± 0.07	58 ± 7.4	21 ± 2.0
Court running sports	Basketball	1.8 ± 0.09	70 ± 7.4	22 ± 1.6
Golf, field, and tennis sports	Golf, field (shotput and discus), and tennis	1.7 ± 0.08	72 ± 17	24 ± 4.9
Intermittent running and jumping sports	Softball and volleyball	1.7 ± 0.07	71 ± 9.9	24 ± 3.2
Field running sports	Rugby and lacrosse	1.7 ± 0.05	65 ± 8.1	24 ± 3.3
Track running sports	Track	1.7 ± 0.06	62 ± 6.8	22 ± 2.1
Mean	**--**	**1.7 ± 0.1**	**66 ± 11**	**23 ± 3.3**

Bone Mineral Density Measurements

We used a portable dual-energy X-ray absorptiometry (DXA) machine, accuDEXA (Schick Technologies, Inc., Island City, NY), to measure BMD (g/cm^2) of the middle phalanx of the third finger on the nondominant hand unless injury had occurred in the third finger, in which we used the other hand. BMD has been shown to be significantly higher in the dominant versus the nondominant hand (Jones and Davie, 1998), likely due to increased use. We used BMD in this study because it has been correlated with whole bone strength (Sievänen, 2000). DXA also measures both cortical and trabecular bone, in which trabecular bone has a higher turnover rate compared to cortical bone (Carbon, 1992). Trabecular bone can be influenced by more recent changes in diet or exercise compared to cortical bone and therefore, DXA readings may also be sensitive to such changes. Additionally, phalangeal BMD has been associated with hip and spinal BMD (Patel et al., 2010) which are significant indicators of total BMD. While phalangeal BMD has been used in limited studies, it is much more time- and cost-efficient and also less invasive than measuring total BMD.

Questionnaires

Using a history questionnaire, we gathered information from each participant about possible covariates of BMD, including sport, any previous injuries, and individual history of vitamin deficiencies, osteopenia, oral contraceptive use and first menarche age (13 ± 1.5 years; Appendix 1). We assessed dietary intake of Omega-3 PUFAs using a 36-item food frequency questionnaire (FFQ) containing foods with Omega-3 PUFAs, e.g. fatty fish, nuts, oils, and meat (Appendices 2 and 3), as dietary Omega-3 PUFA intake influences circulatory Omega-3 PUFAs (Brasitus *et al.*, 1995).

Determining dietary intake can be difficult and no matter the form of assessment including a FFQ, dietary journal, or three-day recall, some sort of bias is present, e.g. under-reporting or misinterpretation of portion sizes. We used a FFQ because it is the most time-efficient method and it evaluates a participant's dietary habits over an extended period of time; a three-day recall or 24-hour questionnaire is more likely to miss large sources of Omega-3 PUFAs, like fatty fish, which are recommended to be eaten only twice a week.

We used the Nutrition Data System for Research (NDSR) from the University of Minnesota to identify quantities of five Omega-3 PUFAs in one serving of every food item. NDSR has been developed and is maintained on the basis of various publications containing standardized procedures for quantification of dietary information with minimal missing nutrient values (Dennis *et al.*, 1980; Schakel *et al.*, 1988; Schakel *et al.*, 1997). The Omega-3 PUFAs had to be differentiated from the Omega-6 PUFA component for nutritional values in NDSR. PUFAs that have the same number of carbons and double bonds can be Omega-3 or Omega-6 depending on the carbon number that double bonds begin. The double bonds in Omega-3 PUFAs begin on the third carbon from the end of the hydrocarbon tail, whereas the double bonds in Omega-6 PUFAs begin on the sixth carbon from the end of the hydrocarbon tail. However, α-linolenic acid (ALA; PUFA 18:3n-3) was not completely differentiated from the Omega-6 counterpart, so we observed slightly elevated values for this Omega-3 PUFA and thus, also for total dietary Omega-3 PUFAs. The other four PUFAs in NDSR that had been differentiated for the Omega-3 PUFA component included parinaric acid (PUFA 18:4n-3), eicosapentaenoic acid (EPA; PUFA 20:5n-3); docosapentaenoic acid (DPA; PUFA 22:5n-3), and docosahexaenoic acid (DHA; PUFA 22:6n-3).

We multiplied values of each PUFA found within one serving of each food by the number of servings per year of that food item for each subject to calculate average daily values (Appendices 2 and 3). For those who reported use of fish oil supplements (n = 7), we used EPA and DHA quantities listed on the nutrient labels for each brand of supplement as these values are highly variable by brand and because it is possible for supplements to make up the majority of one's Omega-3 PUFA intake. We calculated an average daily level for each of the five Omega-3 PUFAs for every subject and also totaled these five average daily Omega-3 PUFA levels to find the total average daily intake of Omega-3 PUFAs for each subject. However, because parinaric acid was not included in in the circulatory results, statistical analyses excluded parinaric acid.

Fatty Acid Analysis

We collected blood from 23 subjects and immediately centrifuged samples at 1000 revolutions per minute (RPM) for eight minutes in ethylenediaminetetraacetic acid (EDTA) tubes using the Centra CL2 centrifuge (Thermo Scientific International Equipment Company, Needham, MA). We isolated plasma from red blood cells (RBCs) and stored plasma samples in Eppendorf tubes at -80°F. RBCs were stored in EDTA tubes at -80°F until shipped to OmegaQuant, Sioux Falls, SD for analysis of 24 fatty acids using gas chromatography. Gas chromatography followed procedures from Harris Scientific, Inc. (2011), which were developed from Morrison and Smith (1964). RBCs were measured because they are a better predictor of long-term dietary intake of Omega-3 PUFAs than plasma (Dougherty *et al*, 1987).

Statistical Analysis

We used regression analyses to evaluate the relationship between circulatory Omega-3 PUFAs and dietary Omega-3 PUFAs as well as circulatory Omega-3 PUFAs with BMD. We used one-tailed Student's t-tests to determine whether BMD or circulatory Omega-3 PUFAs were significantly decreased or increased in individuals with overuse injuries. We also used power analysis for the Student's t-test to determine if our sample size was large enough to detect a difference in means of BMD between those who had history of an overuse injury and of those who had no history. Additionally, we used Chi-square analysis to examine if the number athletes who ever had an overuse injury was different among sport categories as well as an Analysis of Variance (ANOVA) with Tukeys HSD test to compare differences in BMD among each sport category.

Results

In this study, 48% (60 of 125) of athletes had a history of at least one acute injury, whereas 30% (n = 38) had a history of at least one overuse injury. Of the 30% who have had at least one overuse injury, 34% (n = 13) have had tibial/fibular stress fractures, 26% have experienced shin splints (n = 10), and 16% have had knee tendonitis (n = 6; Table 2). The proportions of individuals who had at least one overuse injury were significantly different among each sport category ($p = 4.6 \times 10^{-4}$, Figure 3). The highest proportion and number of athletes who have suffered an overuse injury were found in the cross country running sports, whereas the fewest athletes with overuse injury were found in the field, golf and tennis sports (Figure 3). The proportions of athletes who had at least one acute injury were not significantly different than expected across all sports categories ($p = 2.9 \times 10^{-1}$, Figure 4). The highest frequency and amount of individuals with history of acute injury was in the intermittent running and jumping sports (softball and volleyball), and the lowest frequency was again in the field, golf, and tennis sports category (Figure 4).

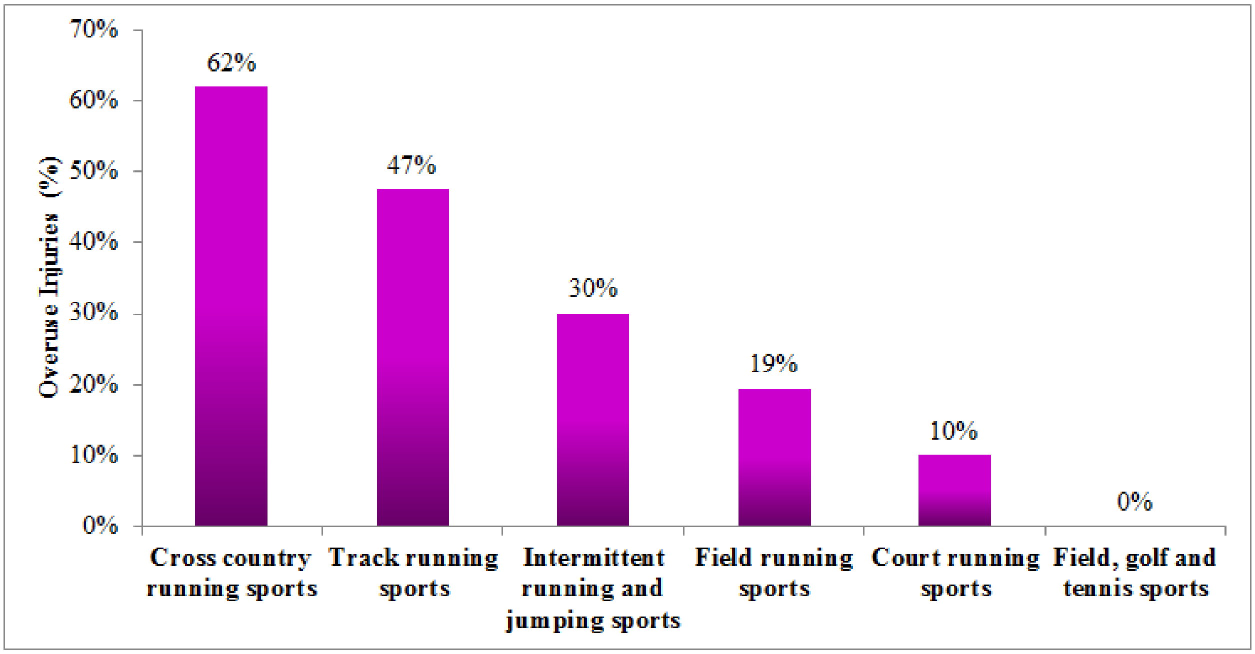


Figure 3. Incidence of at least one overuse injury (%) within each division of sport category. Overuse injury frequency was significantly different across sport categories (Chi-square, $p = 4.6 \times 10^{-4}$).

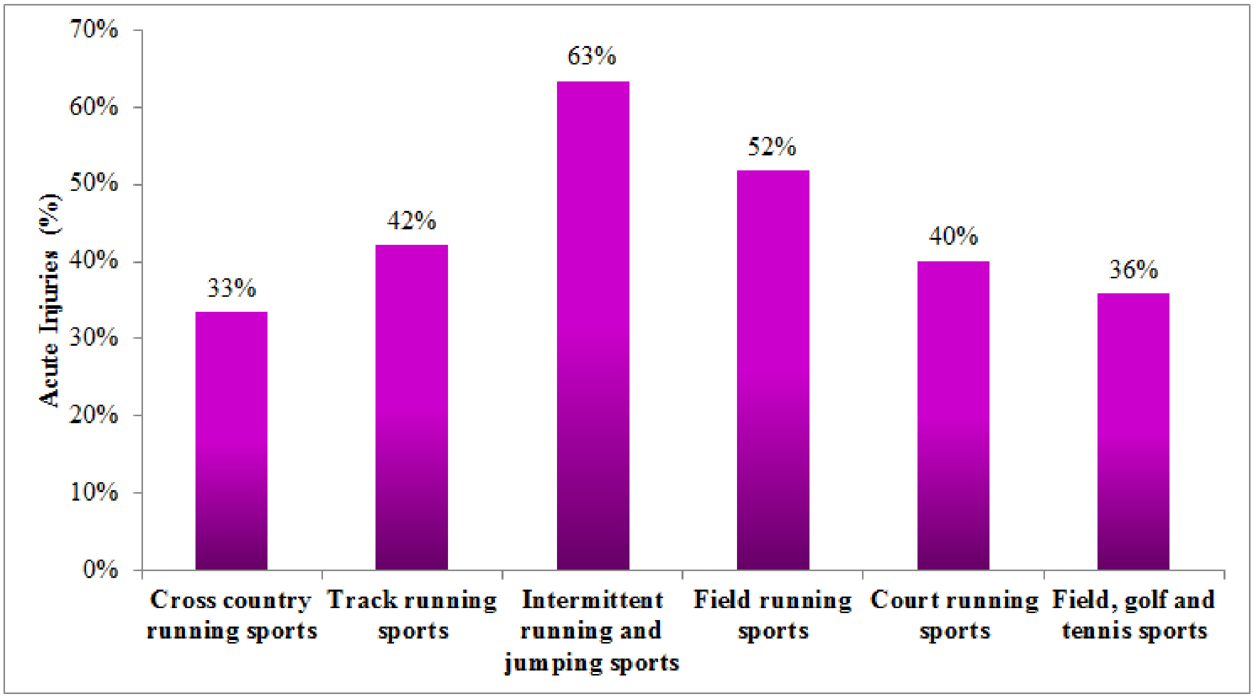


Figure 4. Incidence of at least one acute injury (%) within each sport category. Acute injury frequency was not significantly different across sport categories (Chi-square, 2.9×10^{-1}).

Using the FFQ, we measured absolute amounts of dietary Omega-3 PUFA for 36 food items.

The average dietary, daily intake of Omega-3 PUFAs among our subjects was 1.4 ± 1.1g

(Table 2). The most common sources of dietary Omega-3 PUFAs among all participants came from chicken, butter and turkey. The largest percentage of Omega-3 PUFAs in individual subjects' diets came from vegetable oil, fish oil supplements, and chicken.

Table 2. Mean daily Omega-3 PUFA intake (g) of all participants ± one standard deviation.

Omega-3 PUFA	**Average g/day**
18:3 α-linolenic acid	1.2 ± 1.0
18:4 parinaric acid	$1.6\text{x}10^{-2} \pm 1.4\ \text{x}10^{-2}$
20:5 EPA	$7.5\text{x}10^{-2} \pm 1.2\ \text{x}10^{-1}$
22:5 DPA	$10 \pm 6.2\ \text{x}10^{-2}$
22:6 DHA	$1.4\ \text{x}10^{-1} \pm 1.1\ \text{x}10^{-1}$
Mean of total Omega-3 PUFAs	**1.4 ± 1.1**

We first examined the extent that the FFQ accurately reflected circulating Omega-3 PUFA values. We then examined the effects of circulating levels of Omega-3 PUFA values to factors of BMD in this study (Omega-3 PUFAs and sport category) before observing how all of these factors may contribute to injury risk.

There was no relationship between total dietary (collected from the FFQ) and total circulatory (measured as a percentage of RBCs) Omega-3 PUFA levels ($R^2 = 6.7\text{x}10^{-5}$, $p = 6.36\text{x}10^{-8}$, Figure 5). Regression analysis also revealed no significant relationships between dietary and circulatory values of any single Omega-3 PUFA (e.g. dietary ALA compared to circulatory ALA; $R^2 < 1.0\text{x}10^{-2}$ for all regressions). All further analyses were based only on circulatory Omega-3 PUFAs because we found no significant relationships between dietary and circulatory Omega-3 PUFAs.

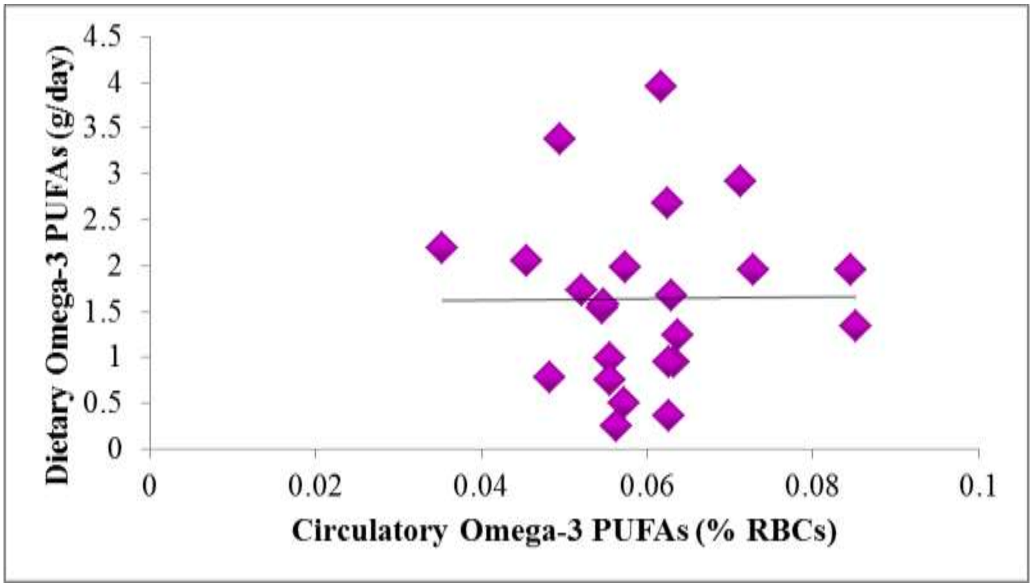


Figure 5. Regression of total dietary Omega-3 PUFAs (g/day) against total circulatory Omega-3 PUFAs (% RBCs) showed no correlation, $R^2 = 6.7\times10^{-5}$, $p = 6.36\times10^{-8}$.

Determinants of Bone Mineral Density

The average phalangeal BMD in this study was $5.42\times10^{-1} \pm 5.33\times10^{-1}$ g/cm^2. Most athletes had similar BMD except court running sports and cross country running sports. The mean BMD in court running sports was significantly higher than in cross country running sports, field running sports, and intermittent running and jumping sports ($p < 0.05$; Figure 6, Table 2). Women's cross country running sports had significantly lower BMD than court running sports, but also in intermittent running and jumping sports, track running sports, and the field, golf and tennis sports category ($p < 0.05$; Figure 6, Table 2). There was no significant relationship between BMD and total circulatory Omega-3 PUFAs ($R^2 = 6.2\times10^{-3}$, $p = 7.2\times10^{-1}$; Figure 7).

Table 2. Mean BMD (g/cm^2) ± one standard deviation across all sport categories.

Sport categories	**BMD (g/cm^2)**
Court running sports	$5.97\times10^{-1} \pm 5.95\times10^{-2}$
Field, golf and tennis sports	$5.58\times10^{-1} \pm 5.10\times10^{-2}$
Track running sports	$5.57\times10^{-1} \pm 6.63\times10^{-2}$
Intermittent running and jumping sports	$5.43\times10^{-1} \pm 5.03\times10^{-2}$
Field running sports	$5.33\times10^{-1} \pm 5.03\times10^{-2}$
Cross country running sports	$5.09\times10^{-1} \pm 4.69\times10^{-2}$

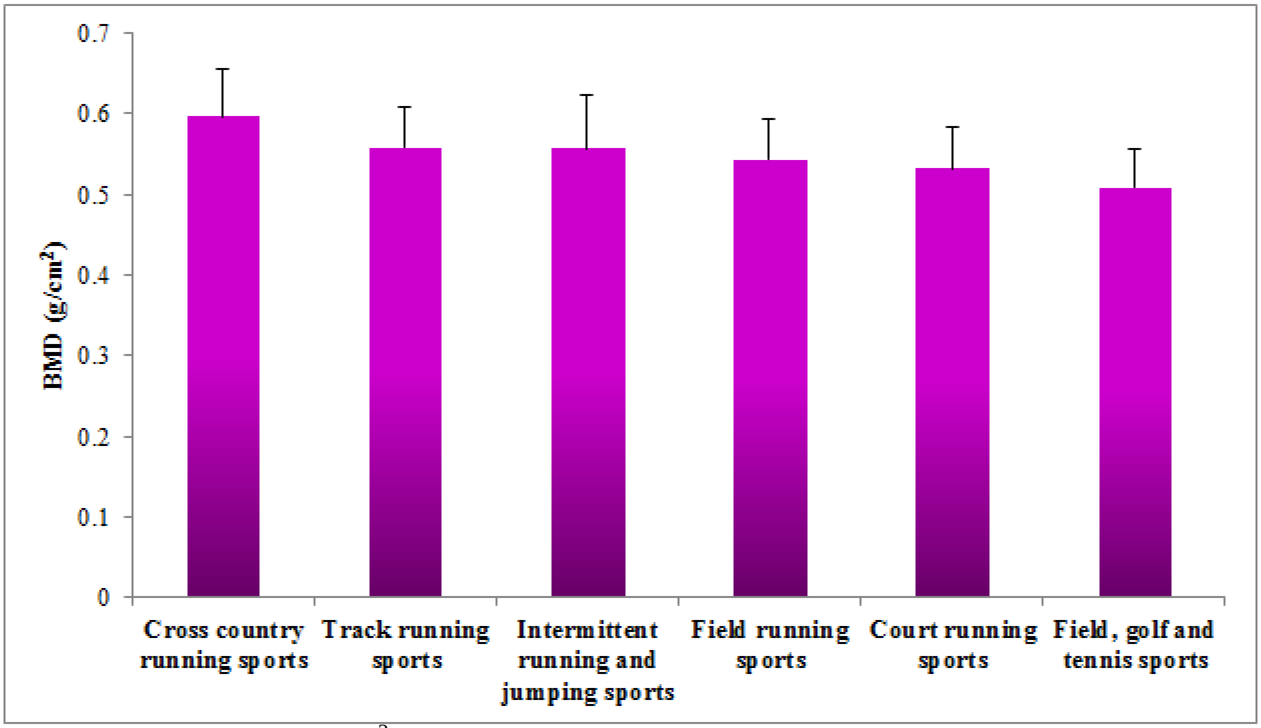


Figure 6. Mean BMD (g/cm^2) in all sport categories + one standard deviation. All pairs Tukeys HSD test showed court running sports had significantly higher mean BMD and cross country running sports had significantly lower mean BMD than other sport categories as described in text ($p < 0.05$).

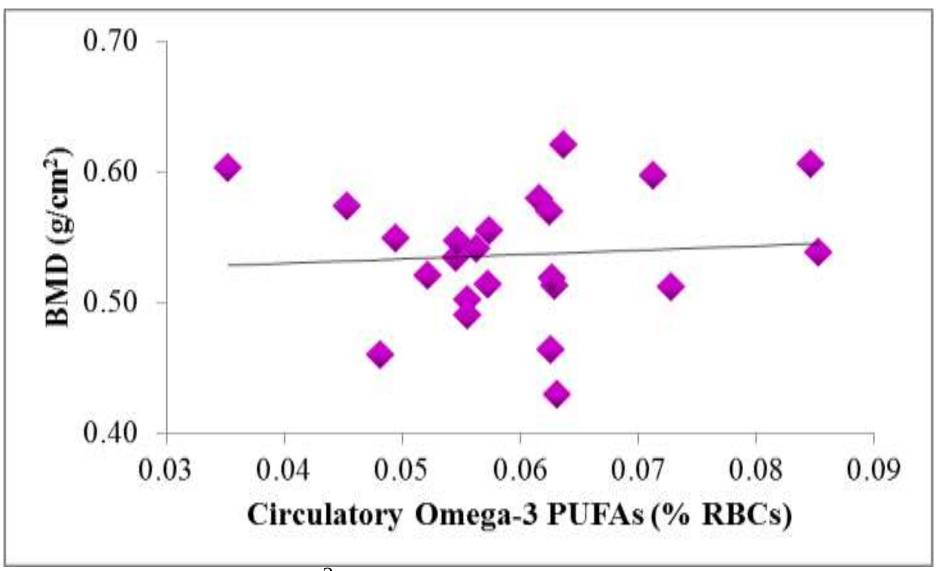


Figure 7. Regression of BMD (g/cm^2) against total circulatory Omega-3 PUFAs (% RBCs) showed no correlation, $R^2 = 6.2 \times 10^{-3}$, $p = 7.2 \times 10^{-1}$.

Due to concern of different training regimens in the category containing field, golf and tennis sports category compared to the other categories, we also ran most analyses without this group. We found no significant differences in any results, with or without the field, golf, and tennis sports category, and therefore, all results include this category.

Determinants of Injury Risk

The mean BMD in those who had no history of an overuse injury ($5.49 \times 10^{-1} \pm 5.9 \times 10^{-2}$ g/cm^2) was significantly higher than in those who had a history of an overuse injury ($5.29 \times 10^{-1} \pm 4.6 \times 10^{-2}$ g/cm^2; $p = 2.0 \times 10^{-2}$; Figure 8). Although the difference between the means is small, power analysis showed that with 95% confidence, we would be able to detect a difference in BMD among those who had no history (n needed ≥ 16; n = 38) and those who did have history of overuse injury (n needed ≥ 35; n = 87).

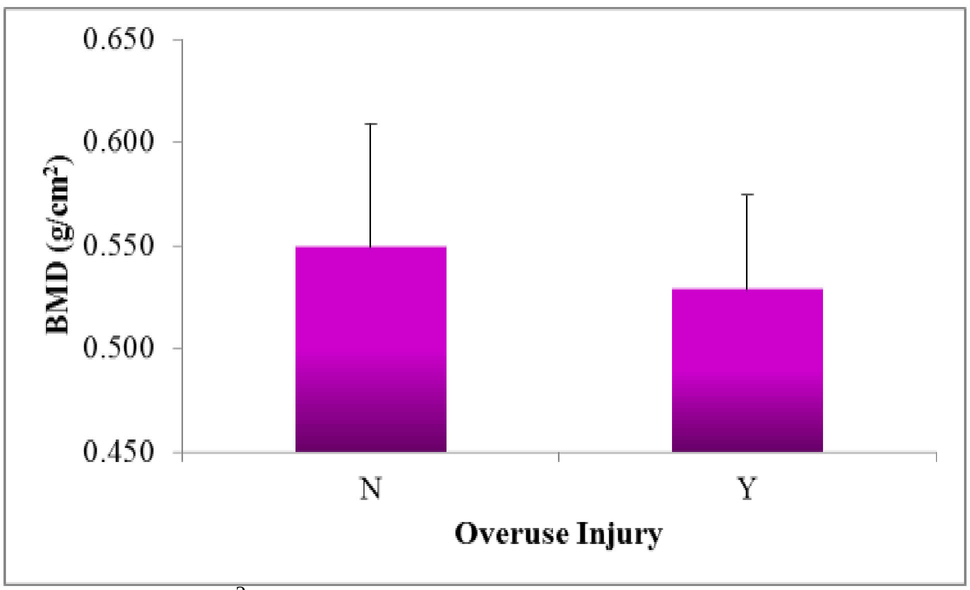


Figure 8. Mean BMD (g/cm^2) + one standard deviation within overuse injury groups. In a one-tailed Student's t-test, $p = 2.0 \times 10^{-2}$.

Circulatory Omega-3 PUFAs appear to be a significant indicator of overuse injuries. The mean of total circulatory Omega-3 PUFAs was significantly higher in those who had a history of overuse injury than in those who had no history of overuse injury ($p = 4.33 \times 10^{-2}$; Figure 9). However, it could be possible that the individuals who have reduced injury rates also happen to take fish oil supplements, therefore influencing the relationship. The mean of all circulatory

Omega-3 PUFAs in subjects taking fish oil supplements (n=3, no history overuse injuries) was $8.04 \pm 7.88\text{x}10^{-3}$%. In subjects not taking fish oil supplements (n=20; n =12 for those with no history of overuse injuries), the mean of total circulatory Omega-3 PUFAs was $5.73 \pm 7.93\text{x}10^{-3}$%).

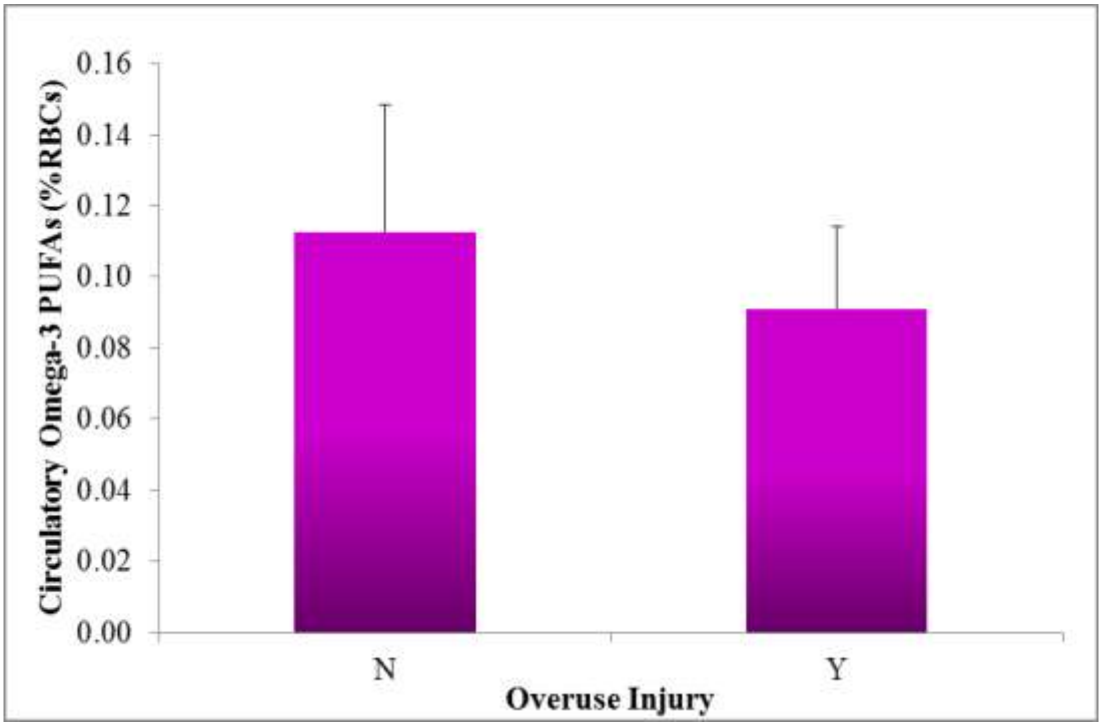


Figure 9. Mean circulatory total Omega-3 PUFAs (% RBCs) between those who have never had an overuse injury and those who had at least one + one standard deviation. A one-tailed Student's t-test showed circulatory Omega-3 PUFAs are significantly higher in those who never had an overuse injury compared to those who had ($p = 4.33\text{x}10^{-2}$).

Discussion

The goal of this study is to identify dietary risk factors of overuse injuries in female athletes, as the incidence of overuse injuries is particularly high in female athletes and military recruits and dietary factors can be easily manipulated. Diet, especially calcium (Dawson-Hughes *et al.*, 1990) and vitamin D, has been shown to be crucial in bone health and preventing osteoporotic fractures in the elderly (Chevalley *et al.*,1994; Sairanen *et al.,* 2000) as well as overuse injuries in athletes (Myburgh *et al.*, 1990; Nieves *et al*, 2010) and military recruits (Lappe *et al.,* 2008). Fats are less understood, however, Omega-3 PUFAs appear to be related to bone health (Järvinen, *et al.,* 2012; Nawata *et al,*. 2013; Rousseau *et al.*, 2009; Terano, 2001) and fracture prevention in the elderly (Orchard *et al.*, 2013). Because no studies have examined the roles of Omega-3 PUFAs on phalangeal BMD in overuse injury risk, we studied these relationships in female collegiate athletes.

In this study, sport category and circulatory Omega-3 PUFAs appeared to be significant indicators of overuse injury risk, whereas sport category and history of overuse injuries were significantly related to BMD. We will first discuss how sport category may influence BMD and how BMD may play a role in injury prevention before examining how Omega-3 PUFAs influence overuse injury risk and musculoskeletal health. We will end in discussion of Omega-3 PUFA levels collected in the FFQ compared to circulatory Omega-3 PUFAs. were consequently not directly tested in any other analyses. This poor relationship may have resulted from shortcomings in the evaluation of dietary Omega-3 PUFAs, in which were not related and factors and suggestions for improvement will also be discussed.

Bone Mineral Density

Specific BMD locations have been related to osteoporotic fracture risk in the elderly and may be a stepping stone for relating BMD in various, easily measured locations to overuse injuries in younger populations. For instance, hip or spinal BMD (Fordham *et al.,* 2000; Miller *et al.,* 2002) and more importantly, BMD at peripheral sites (e.g. finger, hand, wrist, heel, tibia, etc.; Blake *et al.,* 2005; Jones and Davie, 1998; Miller *et al.,* 2002) have been correlated with osteoporotic fractures. Phalangeal BMD has been used in very few studies, but has been correlated with spinal and hip BMD (Patel *et al.,* 2010), the most direct locations for assessing osteoporotic fracture risk in the elderly. This is the first study seeking whether phalangeal BMD can be related to overuse injuries in a younger, athletic population as opposed to osteoporotic fractures in the elderly. We found a significant, negative relationship between phalangeal BMD and overuse injuries, particularly in cross country running sports, who had the highest rate of individuals who had ever had an overuse injury and who also had the lowest mean phalangeal BMD (Figures 3 and 6).

Research shows that sport type can affect BMD differently, e.g. weight-bearing sports tend to increase BMD, thus, sport category may be a confounding variable that we must account for when assessing circulatory Omega-3 PUFAs on BMD status. Court running sports had significantly higher phalangeal BMD than cross country running sports. It is possible that measurement of phalangeal BMD introduced a bias toward increased BMD in court running sports who have increased distal hand use compared to the other athletes, except for track running sports and the field, golf, and tennis sports category (Table 2; Figure 6). On the other hand, cross country running sports had significantly lower BMD than all other sport categories, except for field running sports, agreeing with other studies that repetitive loading may negatively affect bone health (Mudd, 2007; Table 2; Figure 6). If recurring microdamage repeatedly promotes initial stages of the bone formation process (resorption) without completing bone formation, BMD lost during resorption cannot be recovered. We found no other significant differences in BMD among the remaining sport categories (track running sports, intermittent running and jumping sports, field running sports, and the field, golf and tennis sports category), however, the other sport categories had significantly different rates of overuse injury prevalence (Figure 3). Thus, our results may mean that athletes in the other sport categories may experience rigorous training that leads to the formation of microtears that may not severely compromise BMD enough to measure any significant differences. However, these microtears, in combination with total musculoskeletal health (which can affected by other external factors such as fatigue, inflammation, or diet, including the status of circulatory Omega-3 PUFAs), may lead to the significantly different overuse injury rates among sport categories.

Our results suggest that excessive repetitive loading in young female athletes may negatively affect BMD resulting in increased injury risk (Figure 3). Additionally, our results suggest that because BMD was negatively associated with overuse injuries, but BMD was not significantly different among most sport categories although the prevalence of overuse injuries was, then phalangeal BMD alone may be a significant indicator of total BMD and risk for overuse injuries only in cases of extremely decreased BMD. Therefore, several factors may influence the development of overuse injuries in a young population in addition to BMD and exercise regimen (particularly repetitive loading), including musculoskeletal health and diet (Omega-3 PUFAs).

Omega-3 Polyunsaturated Fatty Acids

Our results did not support a relationship between circulatory Omega-3 PUFAs and BMD in collegiate female athletes in this study (Figure 7). However, based on previous studies, Omega-3 PUFAs may be crucial in regulating bone health and also preventing injuries during extreme cases of decreased BMD, as in osteoporosis in the elderly (Orchard *et al.,* 2013) and in repetitive loading in female cross country running sports. Thus, Omega-3 PUFAs may play a larger role in bone health in persons with compromised BMD than in athletes who 1) most likely have not experienced major losses in BMD and who 2) also encounter loading which promotes increased BMD. It is likely that hormonal regulation of BMD is interrupted in the elderly, potentially through increased inflammation and free radicals. Inflammation increases during aging. Specifically, the cytokine (a hormone) interleukin-6 (IL-6), has been shown to have increased production during inflammatory responses in the elderly (Bouchlaka *et al.*, 2010). IL-6 is also a part of the inflammatory process during exercise (Da Silva *et al.,* 2013) and is released by osteoblasts (Ishimi *et al.*, 1990) to promote osteoclastogenesis (Figure 1; Kurihara *et al.*, 1990). Omega-3 PUFAs have been shown to reduce osteoclastic activity indirectly by inhibiting IL-6 (Baghai *et al.*, 2010) and thus, Omega-3 PUFAs may aid potentially compromised cytokine regulation in the elderly to promote bone health. We found that increased levels of Omega-3 PUFAs were associated with decreased risk of overuse injury (Figure 9), but not increased BMD (Figure 7). Our results may suggest that the role of Omega-3 PUFAs on regulating inflammation in favor of improved bone health may also apply to cases of repetitive loading, such as in cross country running sports. Therefore, Omega-3 PUFAs may also regulate other factors involved in musculoskeletal health, in addition to BMD, in favor of injury prevention.

The relationship between circulatory Omega-3 PUFAs and injury prevention may have been influenced by fish oil supplementation in this study, in which the three participants who took fish oil supplements had no history of overuse injury. EPA and DHA, which are found in fish oil, are well known for their anti-inflammatory effects. An inflammatory response occurs as a result of infection or trauma. While trauma may include an acute injury, like a stress or osteoporotic fracture, it may also include rigorous exercise, like repetitive loading. The inflammation process heals damaged tissue by breaking down the damaged tissue and removing debris while promoting factors that aid in tissue repair. Thus, regulatory factors, both pro-inflammatory and anti-inflammatory, are necessary to maintain tissue health. Otherwise, either excessive (or chronic) inflammation or absence of inflammation can lead to damage. Chronic inflammation can lead to excessive breakdown of or become toxic to (through presence of toxic molecules related to oxidative stress, e.g. superoxide, hydrogen peroxide, etc.) tissues such as tendon, bone, and muscle, causing damage, weakness, and overuse injuries. Likewise, no inflammation whatsoever would not allow any healing of the initial trauma.

Although high cumulative doses of exercise have been shown to induce anti-inflammatory effects (Ji *et al.*, 2006), acute (McAnulty, *et al.*, 2005) or rigorous exercise (Armstrong, *et al.*, 2001; McAnulty, *et al.*, 2010) has been found to increase oxidative stress and consequently, free-radical damage. Specifically, reactive oxygen species (ROS) and reactive nitrogen species (RNS) present from oxidative stress can contribute to muscle fatigue (Ferreira and Reid, 2008) which can negatively affect bone health by reducing tolerance to impact (Clansey, 2012), and more specifically, increasing risk for stress fractures. While there are mixed results in human studies (due to variations in subject gender or sport, intensity and duration of activity, as well as amount of Omega-3 PUFAs supplemented), animal studies have been more conclusive in how

Omega-3 PUFAs may aid in regulating multiple inflammatory pathways and cytokines in favor improving bone, muscle, and even tendon health. Omega-3 PUFAs have been shown to reduce oxygen free radicals (Simopoulous, 2007; muscle health) and to regulate cytokines including TNF-α, RANKL, and OPG in favor of upregulating osteoblastogenesis while downregulating osteoclastogenesis (bone health), where decreased TNF-α may also play a role in decreasing major tendon injuries due to inflammation (Scott, 2013).

It is possible that when the effects of Omega-3 PUFAs on bone, muscle, and tendon health are combined, overall risk of overuse injuries may be decreased in athletes who participate in beneficial weight-bearing exercise and whose BMD is not severely compromised. Research in these areas is early in development and further studies are needed to investigate such relationships that may assess: 1) phalangeal, tibial, hip, and spinal BMD; 2) dietary and circulatory measurements of Omega-3 PUFAs and other elements associated with BMD, like vitamin D and calcium; as well as 3) injury rates among large populations, including those prone to overuse injuries (e.g. female distance runners and military recruits), and compared to large populations with low incidences of overuse injuries, such as volleyball and softball players. Additionally, vital inflammatory factors that influence musculoskeletal health could also be measured for comparisons, including by-products of TNF-α, RANKL, OPG, or ROS.

Dietary and Circulatory Omega-3 PUFA Levels

Omega-3 PUFAs collected from the FFQ were not significantly correlated with circulatory Omega-3 PUFA levels (Figure 5). Seventeen of the participants completed two FFQs and there were no significant differences in the dietary Omega-3 PUFA levels collected from these subjects at different times, anywhere from two to six months apart. Because NDSR did not completely differentiate ALA for the Omega-3 component, it is possible that this elevated value may have contributed to our insignificant results. We were not able to collect Omega-6 PUFAs and therefore did not assess Omega-6 PUFAs in this study for methodological reasons.

Future Studies

A sample size larger than 125 participants and in one sport category in a future study may reveal significant relationships, especially between BMD and Omega-3 PUFAs or sport category. Additionally, a larger sample size may better represent each sport category for such analyses, especially golf, field, and tennis sports.

Measuring phalangeal BMD may be an inexpensive, noninvasive, and portable method of predicting fracture risk for the purpose of preventative actions. Use of inactive controls may help determine whether athletes in each sport category have similar BMD which is elevated compared to inactive participants. Such a finding may explain how some studies have recorded that the relative risk associated with phalangeal BMD and osteoporotic fractures may only predict osteoporotic fractures when BMD is extremely low, but not when BMD is only slightly lower than that of the average population. Furthermore, because phalangeal BMD has not been studied as a direct indicator for lower limb overuse injuries, measuring both phalangeal and lower limb BMD may elucidate the association among phalangeal BMD and injuries.

Capturing true dietary intake of any food, but particularly fatty acids, can be difficult, especially in collegiate athletes who are likely not aware of everything they eat nor how much. Because there were no significant differences in the measurements of dietary Omega-3 PUFAs between the two questionnaires completed by the same participants, but measurements were not significantly related to circulatory levels (Figure 5), we believe that the participants answered both questionnaires similarly, but not accurately. Providing more detailed explanations about: 1) the purpose of the questionnaire; 2) the foods involved; 3) the serving sizes of each food group using 3D models instead of pictorial comparisons (e.g. instead of saying a deck of cards is the approximate size of three ounces of meat, have a deck of cards for subjects to see); as well as 4) real life comparisons (a Wendy's burger is about three ounces of beef) could aid in of validation of this FFQ in the future. Additionally, the simple adjustment of increasing the contrast between the rows of food items may increase readability and limit errors in skipped rows. To strengthen any findings, future studies could also quantify calcium and vitamin D intake or supplement use so that these confounding variables can be controlled; they have been widely associated with bone health and may implicate any data related to musculoskeletal health. Studies that capture true dietary intake and that elucidate the wide array of dietary influences in musculoskeletal health can lead to overall increased health and performance in athletes.

Conclusion

Increased circulatory Omega-3 PUFAs and BMD were significantly related to decreased risk of experiencing at least one overuse injury. Omega-3 PUFAs were not related to BMD, suggesting Omega-3 PUFAs may play a larger role in improving musculoskeletal health to reduce injury risk. BMD was significantly higher in court running sports and significant lower in cross country running sports compared to other sports, indicating that the type and amount of load may influence the relationship between BMD and overuse injuries. Measuring phalangeal BMD using a portable accuDEXA increases flexibility to facilitate further studies and is also non-invasive and quick. However, truly clarifying overuse injury risk in athletes may require analyses of not only phalangeal BMD, but also diet and supplements with heavy consideration of confounding variables, particularly sport category and musculoskeletal health. Developing a standardized analysis that considers all mentioned variables may be pivotal for creating a time- and cost-efficient method of assessing injury risk. Research in establishing such a standardized method would allow us to gain a holistic knowledge of injury risk in each individual athlete, in turn leading us to vital preventative measures that could improve short- and long-term health of young athletes including improved musculoskeletal health and reduction of injury and time lost in participation.

Subject #: ______

Appendix 1 – Participant Questionnaire

Thank you for volunteering to participate in this study! The study in which you are about to participate is designed to investigate the effect of fatty acid content and bone mineral density on stress fractures. If at any time you have questions or concerns, please ask one of the researchers and we would be happy to answer your questions.

RESEARCHER USE ONLY

Date: ______________

Subject #: ___________ Height: ________ inches Weight: ________ pounds

BMC: __________ BMD: __________ t-score: __________ z-score: __________

Analysis: __________

Please answer each question to the best of your ability:

1. Sport:

2. Please list any lower limb injuries or surgeries you have ever had and how many:

3. Does anyone in your family have a history of osteopenia or osteoporosis?
   (**circle one**) **Y** **N**

4. Do you have a history of (**circle all that apply**):
    a. Vitamin D deficiency
    b. Calcium deficiency
    c. Osteopenia
    d. Eating disorders
    e. Obesity

6. Are you currently taking oral contraceptives? (**circle one**) **Y** **N**

7. What age were you when had your first menstruation?

8. Are you amenorrheaic (not menstruating/menstruating infrequently)?
    (**circle one**) **Y** **N**

Are you willing to participate in the fatty acid analysis (blood draw)? Y ____ N____

If so, please answer the following questions:

Name: ________________________________ Subject #: ________

Contact Information:

JMU e-mail: ________________________________

Best phone number to reach you: ______________________

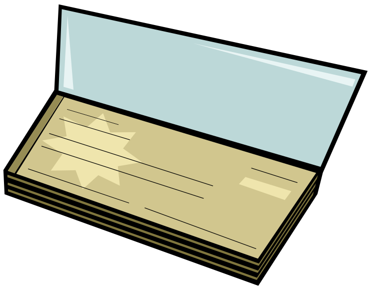
Appendix 2 – Food Frequency Questionnaire for Fatty Acid Content

Please mark the box that best fits. If asked for the number of servings in a category, please specify the number. Serving size is listed under each category—refer to the guide for serving sizes to better estimate number of servings.

	Never	<1 serving a month (please specify number/year)	One serving a month	1-2 servings a week	3-4 servings a week	5-6 servings a week	One serving a day	>1 serving a day (please specify number)
Fish (3 oz.)								
White fish (i.e. Cod, Haddock, Sole, Halibut)								
Oily fish (i.e. Mackerel, Salmon, Trout, Herring [Kippers])								
Sardines								
Tuna:								
–Fresh								
–Canned: light								
–Canned: white (Albacore)								
Shellfish (i.e. Crab, Lobster Prawns, Mussels, Oysters, Shrimp, etc.) (1 oz.)								
Nuts , Seeds (1 oz.) & beans (½ cup)								
Walnuts								
Flaxseeds								
Chia seeds								
Peanuts, pistachios, or almonds								
–Peanut butter (2 tbsp.)								
Other mixed nuts								
Soybeans								
Oils (baking, frying, roasting, grilling or salads) (1 tbsp.)								
Soybean Oil								
Corn Oil								
Safflower Oil								
Vegetable Oil								
Canola Oil								
Flaxseed Oil								
Coconut oil								
Butter								
Margarine								
Lard								
Meat (3 oz)								
Eggs (whole)								
Turkey								
Chicken								
Ground beef								
Pork								
Other meat (i.e. deer, lamb, buffalo)								
Other								
Avocado (1 oz.)								
Olives (5 olives)								
Fish oil supplements –with EPA/DHA								
–without EPA/DHA								
Flax supplements								

Appendix 3 – Serving Sizes

3 oz. fish = checkbook

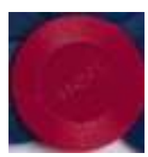


3 oz. meat = deck of cards

½ cup = light bulb

1 oz. = 2 tbsp. = golf ball

1 tbsp. = poker chip



Bibliography

Almeida SA, Trone DW, Leone DM, Shaffer RA, Patheal SL, Long K. Gender differences in musculoskeletal injury rates: A function of symptom reporting? *Med Sci Sports Exerc*. 1999;31(12):1807-1812.

Armstrong III DW, Rue J-H, Wilckens JH, Frassica FJ. Stress fracture injury in young military men and women. *Bone*. 2004;35(3):806-816.

Atteh JO, Leeson S. Effects of dietary saturated or unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. *Poult Sci*. 1984;63(11):2252-2260.

Baek D, Park Y. Association between erythrocyte n-3 polyunsaturated fatty acids and biomarkers of inflammation and oxidative stress in patients with and without depression. *Prostaglandins Leukotrienes Essential Fatty Acids*. 2013;89(5):291-296.

Baghai TC, Varallo-Bedarida G, Born C, et al. Major depressive disorder is associated with cardiovascular risk factors and low omega-3 index. *J Clin Psychiatry*. 2011;72(9):1242-1247.

Beck TJ, Ruff CB, Shaffer RA, Betsinger K, Trone DW, Brodine SK. Stress fracture in military recruits: Gender differences in muscle and bone susceptibility factors. *Bone*. 2000;27(3):437-444.

Bennell KL, Malcolm SA, Wark JD, Brukner PD. Skeletal effects of menstrual disturbances in athletes. *Scandinavian Journal of Medicine and Science in Sports*. 1997;7(5):261-273.

Bhattacharya A, Rahman M, Banu J, et al. Inhibition of osteoporosis in autoimmune disease prone MRL/Mpj-fas lpr mice by N-3 fatty acids. *J Am Coll Nutr*. 2005;24(3):200-209.

Blake GM, Fogelman I. Fracture prediction by bone density measurements at sites other than the fracture site: The contribution of BMD correlation. *Calcif Tissue Int*. 2005;76(4):249-255.

Bloomfield SA, Williams NI, Lamb DR, Jackson RD. Non-weightbearing exercise may increase lumbar spine bone mineral density in healthy postmenopausal women. *American Journal of Physical Medicine and Rehabilitation*. 1993;72(4):204-209.

Borowski LA, Yard EE, Fields SK, Comstock RD. The epidemiology of US high school basketball injuries, 2005-2007. *Am J Sports Med*. 2008;36(12):2328-2335.

Bouchlaka MN, Sckisel GD, Chen M, et al. Aging predisposes to acute inflammatory induced pathology after tumor immunotherapy. *J Exp Med*. 2013;210(11):2223-2237.

Brasitus TA, Davidson NO, Schachter D. Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes. *Biochimica et Biophysica Acta - Biomembranes*. 1985;812(2):460-472.

Carbon R, Sambrook PN, Deakin V, et al. Bone density of elite female athletes with stress fractures. *Med J Aust*. 1990;153(7):373-376.

Chevalley T, Rizzoli R, Nydegger V, et al. Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin-D-replete elderly patients. *Osteoporosis Int*. 1994;4(5):245-252.

Clansey AC, Hanlon M, Wallace ES, Lake MJ. Effects of fatigue on running mechanics associated with tibial stress fracture risk. *Med Sci Sports Exerc*. 2012;44(10):1917-1923.

Coetzee M, Haag M, Joubert AM, Kruger MC. Effects of arachidonic acid, docosahexaenoic acid and prostaglandin E2 on cell proliferation and morphology of MG-63 and MC3T3-E1 osteoblast-like cells. *Prostaglandins Leukotrienes Essential Fatty Acids*. 2007;76(1):35-45.

Coetzer H, Claassen N, Van Papendorp DH, Kruger MC. Calcium transport by isolated brush border and basolateral membrane vesicles: Role of essential fatty acid supplementation. *Prostaglandins Leukotrienes Essential Fatty Acids*. 1994;50(5):257-266.

Da Silva AE, Dos Reis-Neto ET, Da Silva NP, Sato EI. The effect of acute physical exercise on cytokine levels in patients with systemic lupus erythematosus. *Lupus*. 2013;22(14):1479-1483.

Damsgaard CT, Mølgaard C, Matthiessen J, Gyldenløve SN, Lauritzen L. The effects of n-3 long-chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys. *Pediatr Res*. 2012;71(6):713-719.

Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med*. 1990;323(13):878-883.

Dennis B, Ernst N, Hjortland M, Tillotson J, Grambsch V. The NHLBI nutrition data system. *J Am Diet Assoc*. 1980;77(6):641-647.

Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: A study of normal subjects from Italy, Finland, and the USA. *Am J Clin Nutr*. 1987;45(2):443-455.

Eriksson S, Mellström D, Strandvik B. Fatty acid pattern in serum is associated with bone mineralisation in healthy 8-year-old children. *Br J Nutr*. 2009;102(3):407-412.

Fehling PC, Alekel L, Clasey J, Rector A, Stillman RJ. A comparison of bone mineral densities among female athletes in impact loading and active loading sports. *Bone*. 1995;17(3):205-210.

Fernandes G, Lawrence R, Sun D. Protective role of n-3 lipids and soy protein in osteoporosis. *Prostaglandins Leukotrienes Essential Fatty Acids*. 2003;68(6):361-372.

Ferreira LF, Reid MB. Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol*. 2008;104(3):853-860.

Field AE, Gordon CM, Pierce LM, Ramappa A, Kocher MS. Prospective study of physical activity and risk of developing a stress fracture among preadolescent and adolescent girls. *Archives of Pediatrics and Adolescent Medicine*. 2011;165(8):723-728.

Fontani G, Migliorini S. A cognitive approach to physical exercise and sport. *Contemporary Hypnosis and Integrative Therapy*. 2012;29(1):61-77.

Fordham JN, Chinn DJ, Kumar N. Identification of women with reduced bone density at the lumbar spine and femoral neck using BMD at the os calcis. *Osteoporosis Int*. 2000;11(9):797-802.

Frusztajer NT, Dhuper S, Warren MP, Brooks-Gunn J, Fox RP. Nutrition and the incidence of stress fractures in ballet dancers. *Am J Clin Nutr*. 1990;51(5):779-783.

Hay AWM, Hassam AG, Crawford MA, Stevens PA, Mawer EB, Jones FS. Essential fatty acid restriction inhibits vitamin D-dependent calcium absorption. *Lipids*. 1980;15(4):251-254.

Harris WS. The HS-Omega-3 Index Methodological Considerations. 2009-2011. http://omegaquant.com/wp-content/uploads/2013/09/omegaquant_methodological.pdf

Heinonen A, Sievänen H, Kannus P, Oja P, Vuori I. Effects of unilateral strength training and detraining on bone mineral mass and estimated mechanical characteristics of the upper limb bones in young women. *Journal of Bone and Mineral Research*. 1996;11(4):490-501.

Högström M, Nordström P, Nordström A. n-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: The NO2 study. *Am J Clin Nutr*. 2007;85(3):803-807.

Institute of Medicine. Dietary Reference Intakes: Macronutrients. http://www.iom.edu/~/media/Files/Activity%20Files/Nutrition/DRIs/DRI_Macronutrients.pdf

Ishimi Y, Miyaura C, Jin CH, et al. IL-6 is produced by osteoblasts and induces bone resorption. *Journal of Immunology*. 1990;145(10):3297-3303.

Iwamoto J, Takeda T. Stress fractures in athletes: Review of 196 cases. *Journal of Orthopaedic Science*. 2003;8(3):273-278.

Iwamoto J, Takeda T, Sato Y, Matsumoto H. Retrospective case evaluation of gender differences in sports injuries in a Japanese sports medicine clinic. *Gender Medicine*. 2008;5(4):405-414.

Järvinen R, Tuppurainen M, Erkkilä AT, et al. Associations of dietary polyunsaturated fatty acids with bone mineral density in elderly women. *Eur J Clin Nutr*. 2012;66(4):496-503.

Ji LL, Gomez-Cabrera M-, Vina J, eds. *Exercise and Hormesis: Activation of Cellular Antioxidant Signaling Pathway*. ; 2006Annals of the New York Academy of Sciences; No. 1067.

Jones T, Davie MWJ. Bone mineral density at distal forearm can identify patients with osteoporosis at spine or femoral neck. *Br J Rheumatol*. 1998;37(5):539-543.

Kelley DS, Taylor PC, Nelson GJ, Mackey BE. Arachidonic acid supplementation enhances synthesis of eicosanoids without suppressing immune functions in young healthy men. *Lipids*. 1998;33(2):125-130.

Kruger MC, Coetzee M, Haag M, Weiler H. Long-chain polyunsaturated fatty acids: selected mechanisms of action on bone. *Prog Lipid Res*. 2010;49(4):438-449.

Kurihara N, Bertolini D, Suda T, Akiyama Y, Roodman GD. IL-6 stimulates osteoclast-like multinucleated cell formation in long term human marrow cultures by inducing IL-1 release. *Journal of Immunology*. 1990;144(11):4226-4230.

Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL. TNF-a induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest*. 2000;106(12):1481-1488.

Lappe JM, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K. Calcium and vitamin D supplementation decreases incidence of stress fractures in female navy recruits. *Journal of Bone and Mineral Research*. 2008;23(5):741-749.

Lappe JM, Stegman MR, Recker RR. The impact of lifestyle factors on stress fractures in female army recruits. *Osteoporosis Int*. 2001;12(1):35-42.

Lauder TD, Dixit S, Pezzin LE, Williams MV, Campbell CS, Davis GD. The relation between stress fractures and bone mineral density: Evidence from active-duty army women. *Arch Phys Med Rehabil*. 2000;81(1):73-79.

León H, Shibata MC, Sivakumaran S, Dorgan M, Chatterley T, Tsuyuki RT. Effect of fish oil on arrhythmias and mortality: Systematic review. *BMJ*. 2008;337.

Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. biochemistry and relation to pathobiology in human diseases. *N Engl J Med*. 1990;323(10):645-655.

Macdonald HM, New SA, Golden MHN, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: Evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. *Am J Clin Nutr*. 2004;79(1):155-165.

Macleod MA, Houston AS, Sanders L, Anagnostopoulos C. Incidence of trauma related stress fractures and shin splints in male and female army recruits: Retrospective case study. *Br Med J*. 1999;318(7175):29.

Martin BR. 1998. Skeletal Tissue Mechanics. New York: Springer.

Martin-Bautista E, Muñoz-Torres M, Fonolla J, Quesada M, Poyatos A, Lopez-Huertas E. Improvement of bone formation biomarkers after 1-year consumption with milk fortified with eicosapentaenoic acid, docosahexaenoic acid, oleic acid, and selected vitamins. *Nutr Res*. 2010;30(5):320-326.

Martínez-Ramírez MJ, Palma S, Martínez-González MA, Delgado-Martínez AD, de la Fuente C, Delgado-Rodríguez M. Dietary fat intake and the risk of osteoporotic fractures in the elderly. *Eur J Clin Nutr*. 2007;61(9):1114-1120.

McAnulty SR, Nieman DC, Fox-Rabinovich M, et al. Effect of n-3 fatty acids and antioxidants on oxidative stress after exercise. *Med Sci Sports Exerc*. 2010;42(9):1704-1711.

Miller PD, Siris ES, Barrett-Connor E, et al. Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: Evidence from the national osteoporosis risk assessment. *Journal of Bone and Mineral Research*. 2002;17(12):2222-2230.

Moon H-, Kim T-, Byun D-, Park Y. Positive correlation between erythrocyte levels of n-3 polyunsaturated fatty acids and bone mass in postmenopausal Korean women with osteoporosis. *Annals of Nutrition and Metabolism*. 2012;60(2):146-153.

Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 1964;5:600-8.

Mudd LM, Fornetti W, Pivarnik JM. Bone mineral density in collegiate female athletes: Comparisons among sports. *Journal of Athletic Training*. 2007;42(3):403-408.

Myburgh KH, Hutchins J, Fataar AB, Hough SF, Noakes TD. Low bone density is an etiologic factor for stress fractures in athletes. *Ann Intern Med*. 1990;113(10):754-759.

Nattiv A. Stress fractures and bone health in track and field athletes. *Journal of Science and Medicine in Sport*. 2000;3(3):268-279.

Nieves JW, Melsop K, Curtis M, et al. Nutritional factors that influence change in bone density and stress fracture risk among young female cross-county runners. *Nutritional Influences on Bone Health*. 2010:145-147.

Orchard TS, Ing SW, Lu B, et al. The association of red blood cell n-3 and n-6 fatty acids with bone mineral density and hip fracture risk in the women's health initiative. *Journal of Bone and Mineral Research*. 2013;28(3):505-515.

Patel R, Blake GM, Panayiotou E, Fogelman I. Clinical evaluation of a phalangeal bone mineral density assessment system. *Journal of Clinical Densitometry*. 2010;13(3):292-300.

Puel C, Mathey J, Agalias A, et al. Dose-response study of effect of oleuropein, an olive oil polyphenol, in an ovariectomy/inflammation experimental model of bone loss in the rat. *Clinical Nutrition*. 2006;25(5):859-868.

Rousseau JH, Kleppinger A, Kenny AM. Self-reported dietary intake of omega-3 fatty acids and association with bone and lower extremity function. *J Am Geriatr Soc*. 2009;57(10):1781-1788.

Sairanen S, Kärkkäinen M, Tähtelä R, et al. Bone mass and markers of bone and calcium metabolism in postmenopausal women treated with 1,25-dihydroxyvitamin D (calcitriol) for four years. *Calcif Tissue Int*. 2000;67(2):122-127.

Salari P, Rezaie A, Larijani B, Abdollahi M. A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. *Medical Science Monitor*. 2008;14(3):RA37-RA44.

Schnackenburg KE, MacDonald HM, Ferber R, Wiley JP, Boyd SK. Bone quality and muscle strength in female athletes with lower limb stress fractures. *Med Sci Sports Exerc*. 2011;43(11):2110-2119.

Scott A. The fundamental role of inflammation in tendon injury. *Curr Med Res Opin*. 2013;29(SUPPL. 2):3-6.

Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc*. 1988;88(10):1268-1271.

Schakel SF, Buzzard IM, Gebhardt SE. Procedures for estimating nutrient values for food composition databases. *Journal of Food Composition and Analysis*. 1997;10(2):102-114.

Seeman E, Allen T. Risk factors for osteoporosis. *Aust N Z J Med*. 1989;19(1):69-75.

Serrano-Martinez M, Palacios M, Martinez-Losa E, et al. A mediterranean dietary style influences TNF-alpha and VCAM-1 coronary blood levels in unstable angina patients. *Eur J Nutr*. 2005;44(6):348-354.

Sievänen H. A physical model for dual-energy X-ray absorptiometry-derived bone mineral density. *Invest Radiol*. 2000;35(5):325-330.

Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. *Cell*. 1997;89(2):309-319.

Simopoulos AP. Omega-3 fatty acids and athletics. *Current Sports Medicine Reports*. 2007;6(4):230-236.

Skuladottir IH, Petursdottir DH, Hardardottir I. The effects of omega-3 polyunsaturated fatty acids on TNF-a and IL-10 secretion by murine peritoneal cells in vitro. *Lipids*. 2007;42(8):699-706.

Snow-Harter C, Bouxsein ML, Lewis BT, Carter DR, Marcus R. Effects of resistance and endurance exercise on bone mineral status of young women: A randomized exercise intervention trial. *Journal of Bone and Mineral Research*. 1992;7(7):761-769.

Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. *Journal of Bone and Mineral Research*. 2003;18(7):1206-1216.

Sun L, Tamaki H, Ishimaru T, et al. Inhibition of osteoporosis due to restricted food intake by the fish oils DHA and EPA and perilla oil in the rat. *Bioscience, Biotechnology and Biochemistry*. 2004;68(12):2613-2615.

Taaffe DR, Snow-Harter C, Connolly DA, Robinson TL, Brown MD, Marcus R. Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. *Journal of Bone and Mineral Research*. 1995;10(4):586-593.

Terano T. Effect of omega-3 polyunsaturated fatty acid ingestion on bone metabolism and osteoporosis. *World Rev Nutr Diet*. 2001;88:141-147.

Thies F, Miles EA, Nebe-von-Caron G, et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids*. 2001;36(11):1183-1193.

Trichopoulou A, Georgiou E, Bassiakos Y, et al. Energy intake and monounsaturated fat in relation to bone mineral density among women and men in greece. *Prev Med*. 1997;26(3):395-400.

Tulloch I, Smellie WS, Buck AC. Evening primrose oil reduces urinary calcium excretion in both normal and hypercalciuric rats. Urol Res. 1994;22(4):227-30.

Välimäki V, Alfthan H, Lehmuskallio E, et al. Risk factors for clinical stress fractures in male military recruits: A prospective cohort study. *Bone*. 2005;37(2):267-273.

Von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H. The effect of dietary ω-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*. 1999;130(7):554-562.

Weiss LA, Barrett-Connor E, Von Mühlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: The rancho bernardo study. *Am J Clin Nutr*. 2005;81(4):934-938.

Yang J, Tibbetts AS, Covassin T, Cheng G, Nayar S, Heiden E. Epidemiology of overuse and acute injuries among competitive collegiate athletes. *Journal of Athletic Training*. 2012;47(2):198-204.

Zhao Y, Tian Q, Frenkel S, Liu C. The promotion of bone healing by progranulin, a downstream molecule of BMP-2, through interacting with TNF/TNFR signaling. *Biomaterials*. 2013;34(27):6412-6421.