Summary

3 annotations on 3 pages by Richard Ford

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NA). Serum levels of vitamin D, phosphorus, calcium, and estradiol were also collected.

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acids (SFA), only dietary fiber remained negatively associated and % calories from SFA positively associated with lumbar spine BMD. **Conclusions:** Dietary fiber has a significant inverse association and % calories from SFA a positive association with lumbar spine BMD, even after controlling for other nutrient intake and serum levels and

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communities, prevalence studies estimate that up to 16% of female athletes exhibit all three symptoms of the Triad, and the proportion of athletes presenting with any one of the Triad conditions is up to 60% (Gibbs, Williams, & De Souza, 2013). Athletes who restrict dietary intake, exercise for prolonged periods of time, who are vegetarian, and who limit the type of food that they will eat are at increased risk for low EA (Nattiv et al

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Dietary fiber and saturated fat are linked to bone mineral density in amenorrheic athletes

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Thesis

DIETARY FIBER AND SATURATED FAT ARE LINKED TO BONE MINERAL DENSITY IN AMENORRHEIC ATHLETES

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DIETARY FIBER AND SATURATED FAT ARE LINKED TO BONE MINERAL DENSITY IN AMENORRHEIC ATHLETES

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ABSTRACT

The Female Athlete Triad, consisting of the interrelated conditions of low energy availability, leading to menstrual disturbances and low bone mineral density, is commonly diagnosed amongst excessively exercising women. The American College of Sports Medicine emphasizes that the underlying factor of the Triad is a discrepancy between dietary energy intake and the energy requirements needed to support high levels of physical activity in addition to other homeostatic and physiological bodily processes. Although low energy availability is largely recognized as a causative factor for amenorrhea and low bone density, no studies to date have examined specific macro- and micronutrient intake relating to bone mineral density in the female athlete population. The hypothesis to be tested was that a difference in the intake of specific nutrients between athletes with menstrual disturbances (amenorrheic) and regularly menstruating (eumenorrheic) athletes contributes to low bone mineral density in female athletes exhibiting symptoms of the Triad. **Methods:** 4-day food records were collected from 118 females, ages 14-23 years, who exhibited weight within the normal range. 68 participants were amenorrheic athletes (AA), 24 participants were eumenorrheic athletes (EUM), and 26 participants were non-exercising females within the same age group (non-athletes: NA). Serum levels of vitamin D, phosphorus, calcium, and estradiol were also collected. **Results:** In contrast to previous studies, there was no difference in energy availability

between the AA, EUM, and NA groups. The groups did differ in their intake of several macro-and micronutrients, and many of these nutrients correlated significantly with lumbar spine BMD. In a multivariate model that included vegetable and total proteins, soluble, insoluble and total dietary fiber, pectins, phytic acid, natural folate, calcium intake, vitamin D intake, serum vitamin D levels, and % calories from saturated fatty acids (SFA), only dietary fiber remained negatively associated and % calories from SFA positively associated with lumbar spine BMD. **Conclusions:** Dietary fiber has a significant inverse association and % calories from SFA a positive association with lumbar spine BMD, even after controlling for other nutrient intake and serum levels and intake of Vitamin D and calcium. Therefore, fiber and saturated fat may exert effects unrelated to vitamin D status and overall energy availability to impact bone density. Nutrition guidelines for female athlete triad patients need to be reassessed.

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LIST OF ABBREVIATIONS

AA.....Amenorrheic Athlete

ALT.....Alanine Aminotransferase

AST.....Aspartate Aminotransferase

BMD.....Bone Mineral Density

BSI.....Bone Stress Injury

DXA.....Dual X-Ray Absorptiometry

EA.....Energy Availability

EUM.....Eumenorrheic Athlete

FA.....Fatty Acids

FSH.....Follicle Stimulating Hormone

KCAL.....Kilocalories

MET.....Metabolic Equivalent of Task

NA.....Non-Athlete Control

PTH.....Parathyroid Hormone

RER.....Resting Energy Expenditure

SFA.....Saturated Fatty Acids

TSH.....Thyroid Stimulating Hormone

INTRODUCTION

Endurance Athletes at Risk for the Triad

Female high school and college-age endurance athletes are a high-risk population for the "Female Athlete Triad," a syndrome which results in numerous physiological consequences related to low energy availability. Energy availability represents the calories available to perform the physiological functions of the body, including circulation, basic cell maintenance, somatic growth, immune function, reproduction, and many others (Wade & Jones, 2004). Thus, a low energy availability (EA) calculated as dietary intake (EI) minus exercise energy expenditure (EEE) normalized to fat free mass (FFM), results in the inability of the body to perform all of its physiological functions at an optimal level. Compensatory mechanisms that work to restore energy balance and promote survival allow amenorrheic athletes to maintain a stable weight, while simultaneously enduring health consequences from low energy availability (Nattiv et al., 2007). As awareness of the Triad symptoms has increased in athletic and medical communities, prevalence studies estimate that up to 16% of female athletes exhibit all three symptoms of the Triad, and the proportion of athletes presenting with any one of the Triad conditions is up to 60% (Gibbs, Williams, & De Souza, 2013). Athletes who restrict dietary intake, exercise for prolonged periods of time, who are vegetarian, and who limit the type of food that they will eat are at increased risk for low EA (Nattiv et al., 2007). Athletes participating in aesthetic or endurance sports have an especially high risk for low EA, as they participate in a sport where low weight is idealized for peak performance (Sundgot-Borgen, 1994).The prevalence of female athletes demonstrating

one of more Triad elements is concerning considering that low energy availability, loss of menses (amenorrhea), and low BMD resulting from reproductive and metabolic suppression contribute to numerous other compromises to an athlete's performance and overall health.

Amenorrheic Athletes at Risk for Low Bone Mineral Density

Weight bearing physical activity (such as running) is a beneficial mechanical stress on the body that stimulates formation of collagen in bone and the deposition of mineral salts on this collagen framework (Lambrinoudaki & Papadimitriou, 2010). However, the positive effect of mechanical stress on bone is disrupted by the hormonal and nutritional effects of the Female Athlete Triad. Exercise related menstrual dysfunction is associated with conditions of compromised bone health, as it is estimated that 20-50% of physically active women have low bone mineral density (BMD), 10-13% have osteoporosis, and stress fracture prevalence is high (Cialdella-Kam et al., 2014). Ackerman et al. found that while weight-bearing activity increases bone mineral density, increased stress fractures risk and differences in bone strength and architecture are pronounced in amenorrheic athletes (Ackerman et al., 2014). Studies have shown that amenorrheic athletes have a 10-25% lower BMD in comparison to their eumenorrheic counterparts (Nichols, Rauh, Barrack, & Barkai, 2007). Nichols et al. also proposed that the two possible mechanisms contributing to low BMD in athletes with amenorrhea are low energy availability, causing a decrease in the rate of bone formation, and low estrogen, which increases the rate of bone resorption by enhancing osteoclast activity. However, studies of low energy availability and estrogen relating to bone mineral density

in female endurance athletes have produced inconclusive results. Beals et al. found no associations between low EA, amenorrhea, menstrual irregularity, bone mineral density, or stress fracture incidence in 40 female endurance athletes (Beals, Henderson, & Dorais, 2012). Barrack et al. evaluated the risk factors for the Female Athlete Triad (i.e. low EA, menstrual dysfunction, and low bone mass) and the risk for a Bone Stress Injury (BSI) in physically active women (Barrack et al., 2014). In contrast to the results from Beals et al., the authors discovered that the risk for Bone Stress Injuries (BSI) in female athletes increased by 15-20% for a single risk factor for the Triad, and in athletes with combined risk factors, the BSI risk rose to 30-50%. In a six-month dietary intervention study with trained female endurance athletes, a 360 kcal/day carbohydrate and protein restored menstrual function in amenorrheic athletes, but failed to have an impact on total hip and spine bone mineral density z-scores (Cialdella-Kam et al., 2014). Therefore, an intervention for amenorrheic athletes aimed at increasing energy intake, without considering macro-and micro-nutrients that may be beneficial to bone health, may be insufficient to improve bone mineral density in this population.

Inadequate Macronutrient Intake Compromises Bone Mineral Density

Female athletes with exercise related menstrual dysfunction are also at risk for compromised physiological processes resulting from inadequate macro and micronutrient intakes. Manore et al. asserted that amenorrheic athletes consistently consume less protein than eumenorrheic athletes, especially if they are following a vegetarian or vegan diet (Manore, 2002). In addition to the role of protein in synthesizing muscle and providing energy during exercise, adequate protein is necessary for healthy bone. Amino

acid precursors from dietary protein intake are essential for the maintenance of bone structure, and higher protein intake is associated with higher insulin-like growth factor-1 (IGF-1) levels, which stimulates osteoblast activity and facilitates mineralization of the bone matrix (Darling, Millward, Torgerson, Hewitt, & Lanham-New, 2009). Additional proposed benefits to bone health from protein include the suppression of secretion of parathyroid hormone and the improvement of muscle strength (Mangels, 2014). Despite evidence for the positive contribution of protein intake to an optimal BMD, increased calcium excretion associated with higher protein intakes is concerning (Kerstetter & Allen, 1990). However, studies in vegetarians have shown that an increased intake of protein-rich foods is associated with improved bone health and a lower fracture risk (Thorpe, Knutsen, Beeson, Rajaram, & Fraser, 2008). Therefore, the anabolic effect of protein on bone may depend on the source of the protein (vegetable vs. animal), as well as the diverse characteristics of the studied population. Further studies are needed to determine the effects of protein on bone in female athletes, who have a higher protein requirement than their sedentary counterparts (Beals et al., 2012).

To maintain a low body weight for aesthetic or performance purposes, active women may increase fruit and vegetable intake while decreasing dietary fat intake in an effort to decrease dietary energy intake (Hill and Williams, 2014).Physiological consequences arise when fat intake falls below 15% of total intake, and signifies inadequate intake of essential fatty acids (α-linolenic and linoleic acid) and vitamin E (Manore, 2002). Because mammalian cells lack the enzyme n-*3* desaturase, they cannot convert n-6 (linoleic) to n-3 (α-linolenic) fatty acids. The ingestion of omega-3 fatty acids

(in the form of fish oil), has been shown to change the n-6/n-3 ratio in cell membranes. Higher levels of n-3 inhibit the formation of inflammatory cytokines such as IL-6, IL-1, and TNF-α, which are known to facilitate osteoclastogenesis, and thus n-3 fatty acids may be protective to bone (Salari, Rezaie, Larijani, & Abdollahi, 2008). In general, higher levels of n-3/Polyunsaturated Fatty Acids (PUFA) have a positive effect on bone by inhibiting pro-inflammatory cytokines that break down bone, and by reducing serum calcium excretion (Salari et al., 2008).

Effect of Micronutrient Intake on Bone Metabolism

Several other micronutrients are key contributors to bone metabolism, and inadequate intake of these nutrients could lead female athletes to experience the detrimental effects associated with bone uncoupling. Calcium is the predominant mineral associated with bone health, and is required for normal growth, development, and maintenance of the skeleton (Flynn, 2003). Age, sex, dietary intake, bioavailability of nutrients, and mechanical load placed on the body (i.e. from weight-bearing exercise), can all have an effect on calcium requirements. Calcium is integral to the bone turnover process (i.e. a continual renewal of the skeleton that results from bone resorption followed by bone formation). Because serum calcium is maintained within narrow homeostatic limits, a decrease in serum calcium causes an increase in parathyroid hormone (PTH) secretion. PTH works to release calcium from bone, to reduce urinary calcium excretion from the kidney, and to increase the production of 1,25-dihydroxyvitamin D. If serum calcium increases, it stimulates calcitonin to promote bone formation by the osteoblasts (Flynn, 2003). During the period of growth in adolescence,

adequate calcium intake is critical to maximize bone mineralization for optimal bone mineral density (Greydanus, Omar, & Pratt, 2010). Female athletes with menstrual disturbances often have higher dietary calcium intake than their eumenorrheic counterparts, usually from an increased use of supplements. Because estrogen facilitates calcium deposition in bone, amenorrheic athletes, who exhibit low levels of estrogen, also have associated low bone mineral density (Greydanus et al., 2010). Several studies have demonstrated that adolescents with forearm fractures had low calcium intake and low BMD, with higher calcium intake providing a protective effect against fractures (Ackerman & Misra, 2011). Because female athletes in thin-built sports tend to have the lowest calcium intake (Manore, 2002), it is important to investigate how calcium, and its interaction with other nutrients, contributes to BMD in this population.

Vitamin D plays a large role in musculoskeletal health, and an adequate intake is crucial to optimizing bone mineralization. The major physiological role of vitamin D is to maintain calcium and phosphate homeostasis in the body through a series of mechanisms acting on the kidneys, parathyroid gland, bone, and skeletal muscle. Even though a wide variety of foods contain vitamin D (i.e. fatty fish, eggs, and dairy products), the major source of vitamin D is the interaction of UVB light with the skin (Angeline, Gee, Shindle, Warren, & Rodeo, 2013). The active metabolite of Vitamin D (1,25(OH)D3) is formed in the kidney after hydroxylation of 25(OH)D, the storage form of vitamin D. 1,25(OH)D3 is necessary for intestinal calcium absorption and increasing serum calcium levels. Holick et al. asserted that when the serum levels of Vitamin D fall below 30ng/ml, there is a significant decrease in calcium absorption, which correlates with an increase in

parathyroid hormone (PTH) (Holick, 2007). To compensate for the drop in serum calcium, PTH augments the tubular resorption of calcium, and stimulates the kidneys to produce more 1,25-dihydroxyvitamin D to increase intestinal calcium absorption. An additional role of PTH is to stimulate osteoclast activity, which dissolves the mineralized collagen matrix in bone. This overproduction of PTH from low vitamin D status could potentially lead to low bone mineral density and stress fracture.

Previous studies indicate that female athletes demonstrate a high prevalence of stress fracture during adolescence, especially if they participate in high-impact sports (Field, Gordon, Pierce, Ramappa, & Kocher, 2011). The current literature on the efficacy of vitamin D supplements in protecting against stress fractures and low bone mineral density is conflicting (Chen et al., 2014). In one study of adolescent female athletes, Sonneville et al. found that girls in the highest quintile of Vitamin D intake have a 52% lower risk of stress fracture injury compared with girls in the lowest quintile, suggesting the vitamin D intake from food and supplement sources is protective against stress fracture. However, girls with similar activity levels in the highest quintile of calcium intake had an increased risk for stress fracture (Sonneville et al., 2012). Therefore, even though numerous studies denote that athletes are at risk for vitamin D deficiency, which has a profoundly negative impact on health and sports performance, the exact mechanism of vitamin D and its interaction with calcium needs to be further examined in the female athlete population.

An additional mineral contributing to bone metabolism is phosphorus, as 80% of total body phosphorus is stored in the bones and teeth. Takeda et al. asserted that

phosphorus deprivation can lead to demineralization of the skeleton, as it causes a release in calcium and phosphate from bone and increased urinary calcium excretion (Takeda, Yamamoto, Yamanaka-Okumura, & Taketani, 2012). Long-standing phosphorus deprivation can lead to osteomalacia (a defect in bone mineralization). However, high levels of phosphorus intake may also lead to increased bone resorption and osteoporosis due to calcification of the vasculature. Diets high in phosphorus and low in calcium are especially detrimental to skeletal health. Compensatory mechanisms aimed at increasing serum calcium to homeostatic levels include stimulation of parathyroid hormone secretion, which in turn increases bone resorption (Takeda et al., 2012). A proper balance of dietary phosphate intake is especially important to the female athlete, who is already at a heightened risk for stress fractures. Previous studies have shown that for every 100mg of phosphorus intake, the risk of fracture increases by 9% (Pinheiro et al., 2009). However, Rauh et al. revealed that 46% of high school female athletes with elevated bone turnover had inadequate phosphorus intake (by RDA standards), as well as exceedingly low calcium intakes (Rauh et al., 2010). Therefore, appropriate phosphate intake for optimal bone homeostasis needs to be further examined in the female athlete population.

Low magnesium intake has been associated with a variety of mechanisms that lead to poor bone health, such as decreased osteoblastic and osteoclastic activity, vitamin D resistance or reduction, alterations in parathyroid hormone, osteopenia, and bone fragility (Orchard et al., 2014). However, the relationship of magnesium to low BMD and fracture risk is still not completely resolved. While low magnesium intake was associated with low BMD and increased fracture risk in postmenopausal women, high doses of acute

magnesium exposure have been shown to lead to low BMD (Orchard et al., 2014). In a study with female endurance runners, Burrows et al. found magnesium to be positively related to femoral head BMD (Burrows, Nevill, Bird, & Simpson, 2003). They speculated that low levels of magnesium intake could downregulate PTH secretion, which would then lead to decreased calcium uptake from decreased 1,25(OH)2 vitamin D production, and have a negative effect on the bone remodeling process. Ilich and Kerstetter asserted that several other vitamins and minerals could be involved in bone metabolism, such as iron, zinc, vitamin K, Vitamin A, and Vitamin C (Ilich & Kerstetter, 2000).

Detrimental Nutrients to Bone Health

In addition to being concerned about deficient intake of nutrients that improve bone health in the female athlete population, it is also important to pay attention to nutrients that may be directly harmful to bone health. Phytic acid has the ability to bind minerals, protein, and starch, which may alter the solubility, functionality, digestion, and absorption of these constituents in food. The main nutrients affected by increased phytic acid intake are zinc, calcium, iron, sodium, magnesium, manganese, and chlorine. As the female athlete population may have a low intake of bone-building nutrients, excess phytic acid intake may cause a further deficiency in these nutrients (Oatway, Vasanthan, & Helm, 2001). Additionally, foods high in oxalic acid (i.e. spinach, rhubarb, Swiss chard, and beet greens) have been associated with a fractional calcium absorption of only 5%. In comparison, foods low in oxalic acid (i.e. broccoli and bok choy) are associated with a fractional calcium absorption of greater than 50% (Mangels, 2014).

Wolf et al. conducted a dietary intervention study with varying proportion of fiber and fat, and found that women with the lowest ratio of fat to fiber had a 19% lower mean fractional calcium absorption than women who consumed diets with the highest ratio of fat to fiber (Wolf et al., 2000). They conjectured that the reduced calcium absorption could be related to increased intestinal transit time. Increased dietary fiber intake may increase the bulk of intestinal contents, which speeds up the transit time of stool, and allows less time for calcium to be absorbed (Wolf et al., 2000).

Dietary Recommendations for Female Athletes

The interactive and synergistic effects of the dietary pattern of the female athlete, in combination with environmental and lifestyle factors, may prove to be a better determinant of bone mineral density than deficiencies of individual nutrients. Current treatment strategies for the correction of nutritional deficiencies and low energy availability of the female athlete triad are to increase energy intake, reduce physical activity, or a combination of the two treatments (Mountjoy et al., 2014). A recent consensus statement by the International Olympic committee indicated that several studies achieved beneficial results from adding an energy-rich supplement to the athlete's diet, in additional to the implementation of a rest day in the athlete's training regimen. However, they stated that because not all study interventions were successful with the aforementioned treatment strategy, it is necessary to develop a nutritional plan that not only increases overall energy intake, but also takes into consideration specific dietary and psychological factors (Mountjoy et al., 2014).

Objective

This study was the first to relate nutrient intakes of adolescent amenorrheic and eumenorrheic athletes and non-athlete controls, and assess their individual contributions to bone mineral density. By analyzing dietary intake from 4-day food diaries, the differences between the three groups in dietary energy intake, energy availability, and the intake of specific macro and micronutrients were determined. The next aim was to establish if these differences related to bone mineral density z-scores and serum levels of vitamin D, calcium, and phosphorus. It was hypothesized that amenorrheic athletes with low energy availability also have a lower intake of nutrients established to be positive contributors to bone metabolism (i.e. vitamin D, calcium, magnesium) in comparison to their eumenorrheic counterparts. The disparities in dietary patterns between amenorrheic and eumenorrheic athletes may explain how inadequate nutrition negatively impacts bone metabolism, and leads to the low bone mineral density and stress fractures seen in female athletes with low energy availability and menstrual dysfunction.

Table 1. Subject Characteristics and Body Composition

Demographic Data							
	AA (n=68)	**EA (n=24)**	**NA (n=26)**	**ANOVA**	**AA /EA**	**AA/ NA**	**EA/NA**
	Mean ±SD	Mean ±SD	Mean ±SD		p	p	p
Age (years)	19.65 ±2.58	18.21 ±2.88	19.64 ± 2.18	**0.04**	**0.03**	0.1	0.092
Weight (kg)	57.06 ±8.65	58.88 ±7.27	57.15 ± 8.16	0.17	0.16	0.1	0.29
Height (cm)	165.12 ±6.43	165.31 ±7.70	161.72 ± 7.00	0.08	0.99	0.08	0.15
Temp (F)	97.91 ± 0.65	98.23 ± 0.70	98.29 ± 0.94	**0.04**	0.16	0.06	0.95
BMI	20.87 ±2.42	22.21 ±2.60	21.82 ± 2.52	**0.04**	0.06	0.22	0.84
EA (kcal kg $^{-1}$FFM^{-1})	18.36 ±21.63	7.26 ±24.84	19.68 ± 23.58	0.1	0.11	0.97	0.15
REE (kcal)	1255 ± 243	1384 ± 212	1227 ± 201	**0.03**	**0.05**	0.86	**0.05**
% Body fat	23.26 ±4.84	23.58 ±4.17	28.12 ± 6.15	**0.0002**	0.96	**0.00**	**0.01**
Total Lean Mass (kg)	42.35 ±5.51	45.56 ±7.40	39.52 ± 4.65	**0.002**	**0.05**	0.09	**0.00**
Total Fat Mass (kg)	13.62 ±3.89	14.98 ±4.48	16.57 ± 5.46	**0.02**	0.40	**0.01**	0.42
Avg Bouchard EE	2742 ± 631	2771 ± 483	2416 ± 519	**0.04**	0.98	**0.05**	0.10
Lumbar BMD Z-Scores	-0.66 ±1.21	.03± 0.95	-0.39 ± 1.03	**0.04**	**0.02**	0.55	0.40
Hip BMD Z-Score	0.09 ±1.03	.87± 0.99	-0.27 ±0.69	**0.001**	**0.001**	0.23	**0.008**

Mean differences between groups assessed with ANOVA and Tukey's test for normally distributed variables; Kruskal-Wallis and Steel Dwass all-pairs test were applied for non-parametric variables
EA: Energy Availability in kcal per kg of fat free mass (FFM); EA= (Energy Intake – Exercise Energy Expenditure)/FFM
REE: Resting Energy Expenditure in kcal
Avg Bouchard EE: average energy expenditure in kcal from 3-day Bouchard activity record

METHODS

Participant Selection

118 female participants, between 14 and 23 years old, were enrolled to participate in the Female Athlete Study in the adolescent Neuroendocrine Unit of Massachusetts General Hospital. Participants were recruited from local medical clinics, through contact with area coaches and advertising through social media and postings around the Boston area. Sixty-eight athletes were amenorrheic (AA), 24 athletes were eumenorrheic (EUM), and 26 participants were non-athletes (NA). The inclusion criteria for the study were a BMI between the 10^{th} and 90^{th} percentile and a bone age of ≥ 14 years (because 98% of adult height is reached at a bone age of 14 years). Athletes were categorized as amenorrheic if they had absence of menses for ≥ three months within a period of oligomenorrhea (cycle length > six weeks) for ≥ six months, or absence of menarche at ≥15 years. Athletes and controls were categorized as eumenorrheic if they had ≥ nine menses (cycle length 21-35 days) in preceding year. Participants in the athlete groups were classified as "endurance athletes," if they were engaged in ≥ 4 hours of aerobic weight-bearing training of the legs or specific endurance training weekly, or ≥ 20 miles of running weekly for a period of ≥ 6 months in the last year. Non-athletes could not engage in more than 2 hours of weight bearing activity per week, and could not be involved in any team sports. Exclusion criteria for the study included use of medications that affect bone metabolism, conditions other than endurance training that may cause

amenorrhea or low bone mineral density, as well as high or low thyroid-stimulating hormone, high follicle stimulating hormone, or a low hematocrit (<30%).

Screening

Eligibility for the study was assessed, and consent (if the participant is ≥18) or assent (if the participant is ≤18) was obtained at the screening visit. Eligibility was determined through a complete physical and medical history, which included measurements of height, weight, BMI, waist, and hip circumference. Blood was drawn for a complete blood count, and to assess ALT, AST, TSH, FSH, calcium, phosphorus, estradiol, and 25(OH) vitamin D levels. Other potential causes of amenorrhea were ruled out. The Bouchard 3-day activity record was completed, for estimates of physical activity and energy expenditure. The tool qualified 15 minute periods over three days in terms of energy costs on a 1 to 9 scale, which corresponds to 1.0 to 7.8 METs. Reliability within 61 subjects confirmed the Bouchard record to be a highly reproducible tool, with an interclass correlation of 0.96 for mean kcal energy expenditure over 3 days (Bouchard et al., 1983). An x-ray of the risk and hand was taken to determine bone age, and bone density was determined using dual-energy x-ray absorptiometry (DXA). Once the subjects were deemed eligible for participation, they returned to the lab for a baseline visit, at which a medical history was obtained and a physical examination was performed, including assessment of anthropometric measures. The subjects also completed a 4-day food diary to assess dietary intake. In a comparison of the various methods of dietary intake assessment, the 4-day food records had a higher correlation to the estimates of food consumption than 24 hour recall and a detailed quantitative two month history (Morgan et al., 1978).

Analysis

4-day food records for each participant at her baseline visit were analyzed with software at the Bionutrition department of the Clinical Research Center at Massachusetts General Hospital. The dietary intake reports included averages of total intake, and intake of macronutrients and micronutrients. Supplement intake was recorded and added to the corresponding nutrient totals from food intake. Anthropometric, nutrition, and DEXA bone mineral density data were entered into *JMP statistical software* for analysis. First, any statistically significant differences between the AA, EUM, and NA groups in macronutrient and micronutrient intake and energy availability were examined. Energy availability was calculated by normalizing energy intake (EI) – exercise energy expenditure (EEE) to fat free mass (FFM) (i.e. EA=(EI-EEE)/FFM). Mean differences between groups were assessed with the ANOVA and Tukey's test for normally distributed variables, and by applying the Kruskal-Wallis and Steel Dwass all-pairs test for the non-parametric variables. Next, we aimed to determine the relationship between the nutrients and bone mineral density Z-scores (determined by DXA) of the lumbar spine, hip, and whole body. Pairwise correlations were performed for the normally distributed variables, and Spearman correlations for the non-parametric variables. The correlations were analyzed by group (AA, EUM, and NA) and for all groups together. In order to determine the association of specific nutrients with serum levels of vitamin D, calcium, and phosphorus, correlational analysis was performed with the use of the pairwise or Spearman ρ methods, again by group and with all groups taken together. The final aim was to establish whether any nutrients could be significant predictors of bone

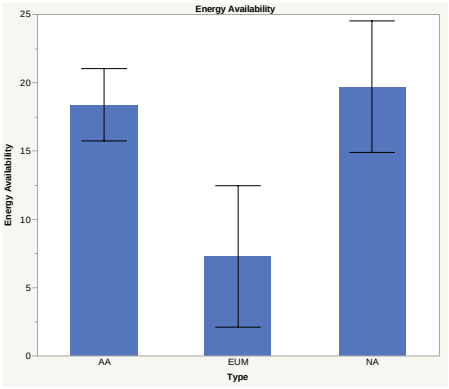
mineral density after controlling for potential confounders. A multi-variate analysis of selected nutrients was performed to assess if they maintained the association with bone mineral density Z-scores when controlling for intake of vitamin D, calcium, protein, and serum vitamin D levels. The selected nutrients differed across groups and had a significant association with bone mineral density.

RESULTS

Participant characteristics

Baseline characteristics of participants are summarized in **Table 1.** EUM were younger than AA and NA. No differences between groups were observed for height and weight; however, there was a significant difference between all groups in BMI, with AA having a trend for a lower BMI than EUM. AA had lower total fat mass than NA, and AA and EUM had lower percent body fat than NA. NA and AA had significantly less lean body mass than EUM. Energy availability did not differ among the groups (**Figure 1A)**, but EUM had a higher resting energy expenditure (REE) than both AA and NA. The Bouchard record for exercise energy expenditure revealed that AA have higher activity levels than NA, however, activity levels between EUM and NA were not significantly different. As expected, AA had lower hip and whole body, and lumbar spine bone mineral Z-Scores than EUM (**Figure 1B)**. NA had lower hip and whole body BMD z-scores than EUM.

A)



B)

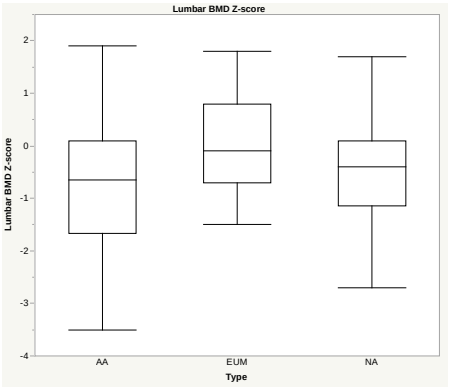


Figure 1: A) No significant difference in energy availability between groups; B) AA has significantly lower lumbar spine BMD than EUM

Macronutrient Analysis

Macronutrient data are shown in Table **2.** Contrary to my hypothesis, there was no difference in daily energy intake between groups. Carbohydrate consumption was not significantly different between groups, however AA consumed food that had a lower glycemic index based on bread and glucose consumption than EUM. AA consumed more vegetable protein and total protein overall than EUM and NA. Total fat intake was not different among groups, however, AA consumed more polyunsaturated fat than the other groups, and a lower percentage of saturated fat than NA. AA had a much larger total, soluble, and insoluble fiber and pectin intake than EUM or NA. Alcohol consumption did not differ among groups, and AA drank significantly more water than EUM or NA. No differences in macronutrient intake between EUM and NA were observed.

Table 2: Macronutrient Intake

	AA (n=68)	**EUM (n=24)**	**NA (n=26)**	**p**	**AA/EUM**	**AA /NA**	**EUM/NA**
Total Energy (kcal)*	2206 ± 717	1918 ± 626	1874 ± 426	0.13	0.38	0.17	0.97
Carbs (g)*	296.5 ± 95.6	266.9 ± 91.3	249.34±63.80	0.10	0.47	0.10	0.72
%Cal Carb	52.5 ± 7.6	54.1 ± 4.8	52.25±6.70	0.57	0.59	0.99	0.61
Protein (g)	96.5 ± 37.4	76.4 ± 25.3	71.9 ±19.1	**0.001**	**0.02**	**0.003**	0.87
% Cal Protein	17.1 ± 4.2	16.2 ± 3.6	15.5 ± 3.1	0.20	0.59	0.20	0.83
Fat (g)*	77.7 ± 35.6	65.5 ± 24.4	66.7 ±21.6	0.34	0.45	0.52	0.97
% Cal Fat	29.8 ± 6.9	29.7 ± 3.8	31.1 ± 5.4	0.65	1.0	0.66	0.72
Animal Protein (g)*	50.5 ± 29.0	44.5 ± 20.9	45.1 ± 15.60	0.76	0.48	0.70	0.79
Vegetable Protein (g)*	45.6 ± 19.2	31.9 ± 12.9	26.8 ± 8.8	**<.0001**	**0.002**	**<.0001**	0.51
SFA (g)*	22.3 ± 11.0	21.8 ± 10.6	23.3 ± 8.7	0.62	0.99	0.65	0.65
%Cal	8.6 ± 2.6	9.8 ± 2.2	10.6 ± 2.6	**0.002**	0.14	**0.002**	0.45

SFA							
Trans FA (g)	2.9 ± 2.3	3.5 ± 2.1	3.6 ± 1.9	0.28	0.50	0.35	0.98
Dietary Fiber (g)*	34.8 ± 15.3	22.5 ± 11.7	17.3 ± 6.6	**<.0001**	**0.0004**	**<.0001**	0.35
Soluble Fiber (g)	8.8 ± 3.5	6.5 ± 2.8	4.8 ± 1.8	**<.0001**	**0.01**	**<.0001**	0.14
Insoluble Fiber (g)	25.4 ± 12.3	15.8 ± 9.1	11.4 ± 5.6	**<.0001**	**0.0006**	**<.0001**	0.31
Pectins (g)	4.9 ± 2.6	3.0 ± 1.5	2.3 ± 0.9	**<.0001**	**0.001**	**<.0001**	0.45

Carbs: Carbohydrates
Cal: Calories
SFA: Saturated Fatty Acids
FA: Fatty Acids
ANOVA used for three group comparisons followed by Tukey's test with EUM as the comparison group
*Values not normally distributed were compared by the Kruskal Wallis Test, followed by the Steel Dwass test with EA as the comparison group

Micronutrient Analysis

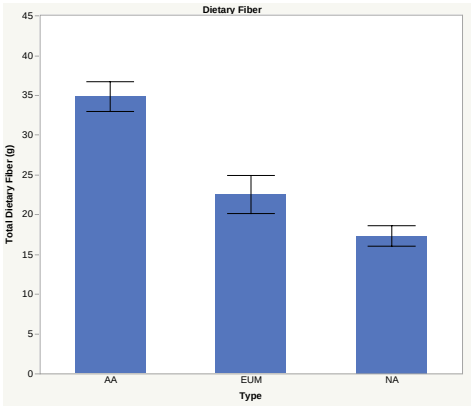
Data regarding micronutrient intake are shown in Table **3.** AA had a higher consumption of the minerals magnesium, phosphorus, zinc, copper, manganese, and potassium than both of the other groups. AA also had higher calcium, folic acid, and iron intake than the NA group. AA demonstrated a much higher vitamin intake, and had significantly higher consumption of Vitamins A, E, and D, beta-carotene, niacin, natural folate, and pantothenic acid than EA or NA. The consumption of several sugar alcohols, including formononetin, erythritol, mannitol, and xylitol, was higher in AA than both other groups, and higher sorbitol intake than NA. AA had higher oxalic acid consumption than NA, and higher phytic acid consumption than both other groups. Intake of two isoflavones, genistein and biochanin A, were higher in the AA than NA groups, and AA had higher daidzein intake than both groups. Interestingly, there was no significant difference in micronutrient intake between EUM or NA groups.

Table 2. Micronutrients

	AA (n=68)	EUM (n=24)	NA (n=26)	ANOVA	AA/ EUM	AA/ NA	EUM/ NA
Phytic Acid (mg)*	1276 ± 621	621 ± 384	700 ± 370	**<.0001**	**0.002**	**<.0001**	0.78
Oxalic Acid (mg)*	541 ± 539	336 ±263	247 ± 288	**<.0001**	0.09	**<.0001**	0.09
Glycemic Index (Glucose) *	56.9 ±3.83	59.71 ± 4.35	59.01 ± 4.49	**0.0012**	**0.0018**	0.06	0.87
Omega-3 FA (g) *	36.6 ± 109.9	12.8 ± 40.4	1.4 ± 0.6	0.14	0.47	0.18	0.89
Vitamin D (IU)	16.57 ± 16.72	8.37 ± 7.18	7.45 ± 7.19	**0.0036**	**0.03**	**0.01**	0.97
Calcium (mg)	1595 ± 781	1218 ± 709	943 ± 394	**0.0002**	0.06	**0.0003**	0.35
Phosphorous (mg)	1597 ± 567	1243 ± 463	1149 ± 336	**0.0002**	**0.01**	**0.0006**	0.79
Magnesium (mg)	499 ± 220	314 ± 122	273 ± 86	**<.0001**	**0.0001**	**<.0001**	0.70
Iron (mg)	36.2 ± 35.9	22.1 ± 16.5	17.8 ± 9.8	**0.01**	0.10	**0.02**	0.86
Vitamin C (mg)	213.5 ± 185.5	119.3 ± 72.3	209.35 ± 233.9	0.06	0.08	0.99	0.19
Folic Acid (mcg)	403.4 ± 329.4	286.7 ± 187.1	247.7 ± 212.6	**0.03**	0.20	**0.05**	0.88
Natural Folate (mcg)*	644.2 ± 328.8	499.4 ± 191.1	439.0 ± 228.1	**<.0001**	0.01	**0.0002**	0.47
Dietary Folate Equivalents*	809.8 ± 449.0	744.9 ± 378.6	596.3 ± 352.8	**0.01**	0.89	**0.01**	0.16

FA: Fatty acid
*Values not normally distributed were compared by the Kruskal Wallis Test, followed by the Steel Dwass test with EUM as the comparison group
ANOVA used for three group comparisons followed by Tukey's test with EUM as the comparison group when ANOVA was significant

A)



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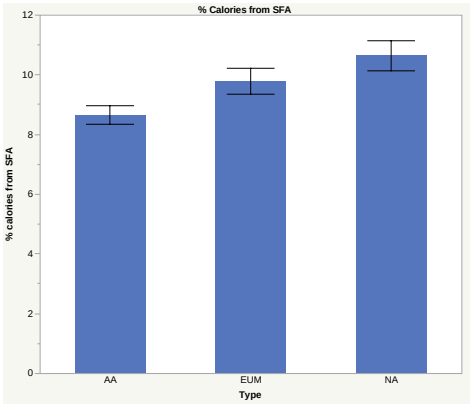

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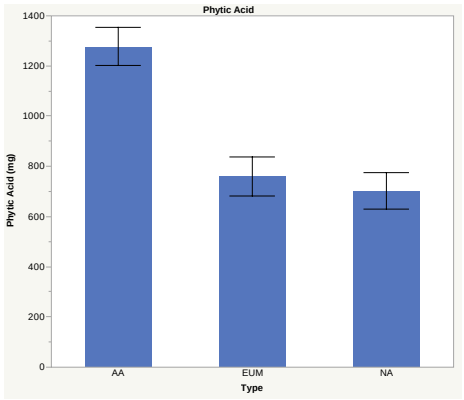

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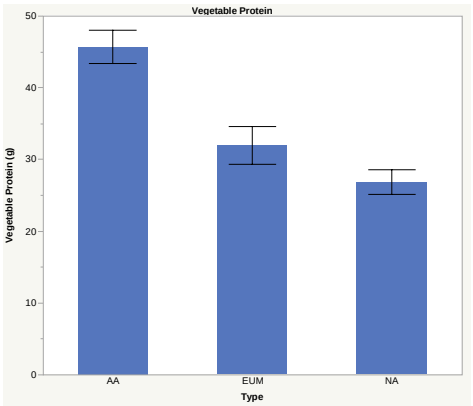


Figure 2: A) AA significantly higher intake of dietary fiber than EUM (p=.0004) and NA (P<.0001) B) AA significantly lower %SFA intake than NA (p=.002); C) AA higher phytic acid intake than EUM (p=.002) and NA (p<.0001); D) AA had higher vegetable protein intake than EUM (p=.002) and NA (p<.0001)

Correlation Analysis between nutrients and serum levels of Vitamin

D, Calcium, and Phosphorus

Many of the micronutrients that differed among groups for intake were also found to have an association with serum levels of vitamin D

(Table **4**). Significant positive correlations were observed for vitamin D levels with intake of magnesium, phosphorus, calcium, vitamin K, vitamin D, folate, components of dietary fiber (total, soluble, insoluble and pectins), and phytic and oxalic acid. A positive trend was seen for vegetable protein and vitamin D levels. AA had a positive association of magnesium and calcium intake and vitamin D levels. No significant associations were observed between the selected micronutrients and serum calcium. NA had a significant positive association between total phosphorus intake and serum phosphorus.

Table 3. Nutrient and Serum Level Correlations

	Vitamin D (ng/ml)		Calcium (mg/dl)		Phosphorus (mg/dl)	
	r	**Prob**	**r**	**Prob**	**r**	**Prob**
Total Dietary Fiber						
AA	0.19	0.14	-0.16	0.21	-0.12	0.34
All	0.33	**0.0005**	0.09	0.35	-0.11	0.25
Total Magnesium						
AA	0.01	**0.04**	-0.05	0.69	-0.03	0.81
All	0.37	**<.0001**	0.12	0.22	-0.06	0.53
Total Phosphorus						
AA	0.18	0.14	-0.13	0.30	0.07	0.56
All	0.25	**0.01**	-0.02	0.86	0.04	0.64
Total Calcium						
AA	0.33	**0.01**	-0.09	0.47	0.04	0.78
All	0.41	**<.0001***	0.07	0.48*	0.01	0.92*
Total Vitamin D						
AA	0.21	0.09	0.02	0.88	0.23	0.06
All	0.33	**0.0005***	0.08	0.41*	0.11	0.26*
Phytic Acid						

AA	0.09	0.50	-0.07	0.58	-0.10	0.45
All	0.29	**0.0024***	0.18	0.06*	-0.04	0.64*
Oxalic Acid						
AA	0.16	0.20	-0.19	0.14	-0.09	0.46
All	0.38	**<.0001***	0.07	0.46*	-0.17	0.08*
Folate						
AA	0.08	0.51	-0.23	0.06	-0.14	0.28
All	0.24	**0.01***	0.06	0.53*	-0.09	0.37*
Natural Folate						
AA	0.19	0.14	-0.14	0.28	-0.09	0.46
All	0.38	**<.0001***	0.07	0.47*	-0.10	0.28*
Vegetable Protein						
AA	0.16	0.21	-0.16	0.20	-0.03	0.81
All	0.26	0.08	0.07	0.44	-0.05	6.13

* Non-parametric Spearman ρ
correlation analysis performed through pairwise (parametric) or Spearman ρ (non-parametric) methods, by group and with all groups taken together

Correlation Analysis between Nutrition and Bone Mineral Density

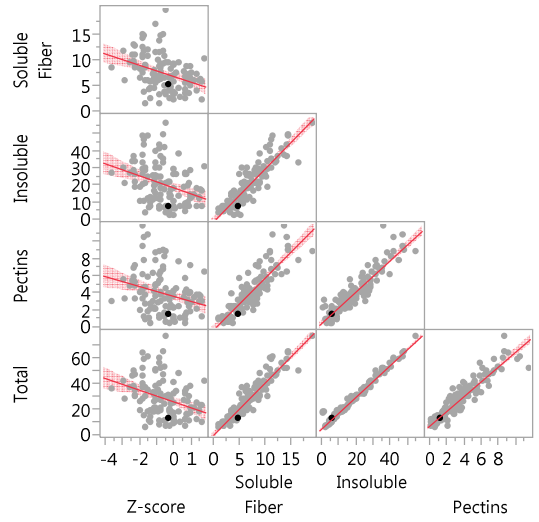
Associations between macro- and micro-nutrients with hip, lumbar spine, and whole body bone mineral density Z-scores are shown in Table **5.** For all groups combined, there was a significant negative association of lumbar BMD with components of dietary fiber (total fiber, soluble fiber, insoluble fiber, and pectins), minerals (magnesium, phosphorus, and calcium), folate, phytic and oxalic acid, total carbohydrates, total protein, vegetable protein, and omega-3 fatty acids. A positive association was observed for bone density with percent calories from saturated fatty acids, trans-fatty acids, and percent calories from animal protein. Both EA and AA had significant negative associations between lumbar BMD Z-scores and fiber (total, soluble, and insoluble), magnesium, phytic acid, folate, and vegetable protein. Only AA had negative associations between oxalic acid and percent vegetable protein. Positive associations were observed in AA between percent animal protein, cholesterol, and percent calories from SFA intake and lumbar BMD.

There were no significant associations observed in the NA group between nutrients and lumbar BMD.

Because dietary fiber intake has been shown to decrease apparent nutrient absorption and increase digestive transit time in the GI tract, we wanted to determine whether the associations of the significant nutrients continued after controlling for total dietary fiber intake. In a multivariate model controlling for calcium, vitamin D, protein, and dietary fiber intake, and serum vitamin D levels, other significant nutrients were added to the analysis model one at a time and persistent significance was determined. The selected nutrients added to the model were those that had demonstrated significant associations with BMD and a significant difference between groups, and included intake of magnesium, phosphorus, phytic acid, folate, percent calories from saturated fatty acids, and percent calories from vegetable protein. The analysis of all groups together revealed that % saturated fatty acids was significantly positively associated with BMD after controlling for total dietary fiber, vitamin D and calcium intake, protein intake, and serum vitamin D levels (PE=117;p=.0178). The only nutrient that remained a significant negative predictor of lumbar spine BMD in this multivariate model was dietary fiber (PE=-.026, p=.0079). The significant negative association with fiber persisted for only the AA when divided into groups (PE=-.026, p=.036). Interestingly, dietary fiber had a significantly positive correlation with serum vitamin D levels, and a negative correlation with lumbar spine BMD. To determine whether participants with a higher fiber intake also had a high supplement of vitamin D intake, a multivariate analysis was created controlling for dietary fiber and vitamin D intake of serum vitamin D levels. The positive

association between dietary fiber and serum vitamin D remained significant (PE=.22, p=.0084), without a significant association for vitamin D intake. Therefore, serum vitamin D status may be primarily determined by sun exposure. For example, AA have a significantly higher fiber intake than EA and NA, and may also spend more time running outside, which would lead to higher serum vitamin D levels.

A)



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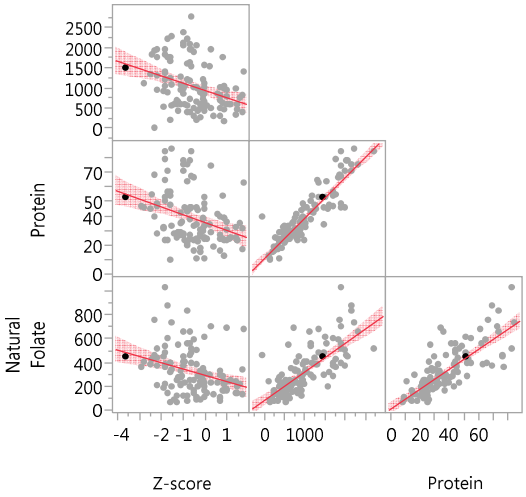

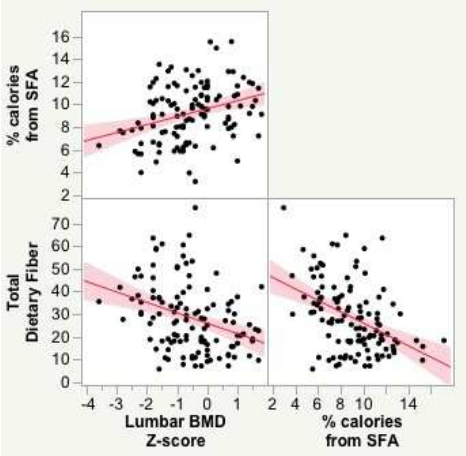

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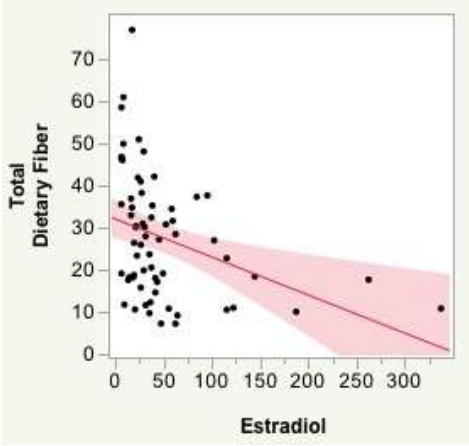

D)

Fig 3: Scatterplot Matix with regression fit line and confidence intervals; A) Components of Dietary Fiber with lumbar BMD; B) Folate, vegetable protein, and natural folate with lumbar spine BMD; C) Correlation between Total Dietary Fiber, % Calories SFA and lumbar Spine BMD;D) Total Dietary Fiber has significant negative association to serum estradiol after controlling for BMI and duration of amenorrhea (PE=-1.18; p=.02)

Table 4. Nutrients and Lumbar Spine BMD

	All Groups		AA	
	r	P-Value	r	p-value
Total Dietary Fiber	-0.35	**0.00**	-0.30	**0.01**
Soluble Fiber	-0.36	**<.0001**	-0.35	**0.00**
Insoluble Fiber	-0.34	**0.00**	-0.29	**0.02**
Pectins	-0.27	**0.00**	-0.22	0.07
Phytic Acid*	-0.37	**<.0001**	-0.29	**0.02**
Oxalic Acid*	-0.25	**0.01**	-0.24	**0.05**
Veg Protein	-0.34	**0.00**	-0.32	**0.01**
%Veg Protein*	-0.25	**0.01**	-0.30	**0.01**
Animal Protein	-0.06	0.50	0.00	0.97
% Animal Protein*	0.25	**0.01**	0.30	**0.01**
%Cal Fat	0.12	0.21	0.05	0.66
% Cal SFA	0.32	**0.00**	0.33	**0.01**
Calcium	-0.21	**0.02**	-0.12	0.33
Vitamin D Total	-0.13	0.15	-0.01	0.91

% Cal: % Calories from
Veg: Vegetable
SFA: Saturated Fatty Acids
correlation analysis performed through pairwise (parametric) or Spearman ρ (non-parametric) methods, for AA and with all groups taken together
* Spearman's Correlation
P-value <.05 after controlling for calcium intake, serum vitamin D (ng/ml), and duration of amenorrhea

DISCUSSION

Adolescent and young adult female athletes are at risk for the Female Athlete Triad, a syndrome connecting low energy availability with menstrual disturbances and low bone mineral density (Gibbs et al., 2013). The syndrome contributes to deterioration of athletic performance and health by increasing the risk for musculoskeletal injuries (Rauh et al., 2010) and stress fractures through hormonal imbalance and interruptions to bone remodeling (Feingold & Hame, 2006) .The consequences of the Triad are thought to originate from low energy availability, which is the failure of the athlete to ingest adequate calories to compensate for energy expended during exercise. Nichols et al. proposed that the two possible mechanisms contributing to low BMD with amenorrhea are low energy availability, decreasing the rate of bone formation, and low estrogen, which increases the rate of bone resorption by enhancing osteoclast activity (Nichols et al., 2007). As previous studies of amenorrhea athlete focus on the maladaptive consequences of low energy availability for BMD, this was the first study to determine if amenorrheic athletes have a deficiency of specific macro-and micro-nutrients that contribute to impaired bone metabolism. Therefore the purpose of this study was to compare overall caloric intake and energy availability between amenorrheic (AA), normally menstruating (EUM), and non-athletes (NA), as well as specific macro-and micronutrients that have an association with bone mineral density.

Surprisingly, there was no difference in energy availability between groups (i.e. energy expenditure subtracted from total energy intake, relative to fat free mass). Because menstrual dysfunction and low bone mineral density, the other two conditions of the Triad, have been linked in to the energy deficiency from low energy availability (Loucks et al., 2007), our data are not consistent with previous studies. Therefore, the lower bone mineral density and menstrual dysfunction observed in the AA group compared to the other two groups may have an underlying cause separate from energy availability. However, AA had a lower resting energy expenditure (REE) (calculated through indirect calorimetry) than EUM, as well as a significantly lower percentage of REE predicted by individual energy requirements. DeSouza et al. found that a low REE is attributed to a state of energy conservation caused by energy deficiency in exercising women (De Souza et al., 2007). Therefore, the hypo-metabolic state in AA, as reflected by the measurements of indirect calorimetry, is in contradiction to our calculations of energy availability, in which there was no difference between groups. In addition to the possibility of reporting bias in the food logs, another possible explanation for this contradiction could be a dietary intake pattern that has a significant effect on nutrient absorption and metabolism, leading to a hypo-metabolic state even when adequate calories are ingested.

The high fiber intake of AA, compared to EA and NA, may partly explain the discrepancy between high energy availability and low REE. Dietary fiber has been shown to affect dietary energy availability and digestibility of complex foods. Baer et al. reported that the fiber content of foods can interact with protein and fat, and decrease the

metabolizable energy (ME) of diet by affecting the digestibility of these components (Baer, Rumpler, Miles, & Fahey, 1997). They also assert that adding up fat, carbohydrate, and protein will not give an adequate estimation of metabolizable energy from caloric value, and that the interaction of these nutrients with dietary fiber needs to be considered. An inverse relationship was found between dietary fiber intake and the ME of diets, when accounting for total dietary fiber as well as insoluble dietary fiber. One mechanism by which fiber decreases the ME of food is its ability to alter upper gastrointestinal function without affecting apparent glucose absorption. Shinnick et al. discovered that an increase in dietary fiber caused a increase in transit time from the mouth to cecum, as well as decreasing the absorption of folate and zinc (Shinnick, Hess, Fischer, & Marlett, 1989).

Contrary to my hypothesis, there was a significant inverse association with magnesium, phosphorus, and calcium with lumbar spine BMD, and no association with dietary vitamin D. Furthermore, AA had significantly higher intake of vitamin D and the aforementioned minerals, yet had a significantly lower bone mineral density than the other two groups. Therefore, the data contradicts previous studies that have shown these nutrients to be beneficial and integral to bone mineralization and the bone turnover process (Flynn, 2003) (Holick, 2007) (Takeda et al., 2012) (Orchard et al., 2014). Interestingly, positive associations were observed for vitamin D levels with intake of calcium, vitamin D, fiber, and phytic acid. However these same nutrients had a negative association to lumbar spine BMD. This implies that serum level of vitamin D may not be an appropriate indicator of bone health, and that the interrelationship between the aforementioned nutrients may have an affect on bone.

Because dietary fiber and % saturated fat were the only significant predictors of lumbar spine after controlling for calcium, vitamin D, and protein intake, and serum vitamin D levels, they may directly affect the absorption of these nutrients.

Wolf et al. also discovered that fractional calcium absorption was inversely associated with dietary fiber intake, and that women in the lowest tertile of the dietary fat/fiber ratio had a 19% lower ratio of fractional calcium absorption than did women in the highest tertile of the dietary fat/fiber ratio (Wolf et al., 2000). Because AA demonstrated a lower saturated fat intake than NA and a higher fiber intake than both groups, they may have a lower fractional absorption of calcium and other nutrients that have anabolic effects on bone. This theory helps explain why AA exhibit lower bone mineral density despite reporting a higher intake of nutrients such as magnesium, phosphorus, vitamin D, and zinc.

Phytic acid intake significantly correlated with fiber intake ($r=.8801$; $p<.001$), and was higher in AA than the other two groups. Phytic acid (PA) has also been shown to exhibit numerous anti-nutritional qualities. In a review of phytic acid metabolism, Oatway et al. discussed the ability of PA to bind to minerals, proteins, and starch, which can affect their solubility, functionality, digestion, and absorption. They noted that minerals and nutrients most affected by PA are calcium, sodium, iron, magnesium, manganese, chorine, zinc, starch, and protein (Oatway et al., 2001). Foods high in PA, such as wheat bran, legumes, seeds, nuts, and soy isolates, may reduce the bioavailability of these nutrients, and prevent their beneficial effects on bone metabolism.

Because dietary fiber remained a significant negative predictor and % saturated fatty acids a positive predictor of lumbar spine BMD, it is possible that the ratio of fiber to fat in dietary intake directly regulates the metabolizable energy and bioavailability of macro- and micro-nutrients involved in bone metabolism, independent of overall caloric intake. However, it may also exert indirect effects on bone metabolism through mechanisms affecting other components of the Triad. A prospective cohort study by Gaskins et al. revealed that dietary fiber consumption was inversely associated with concentrations of estradiol, progesterone, LH, and FSH, and was positively correlated with the risk of anovulation (a menstrual cycle in which the ovaries do not release an oocyte) (Gaskins et al., 2009). A possible mechanism for these hormone alterations was proposed by Aldercreutz, who found that there were positive associations between total fiber and grain fiber intake, and fecal E_1 and E_2 excretions. Additionally, he found that a high fat/fiber ratio has highly significant negative correlations with estrogen excretion (Adlercreutz, 1991). The increased excretion of estrogens with fiber intake is caused by fiber's ability to bind sex hormones, with an especially increased affinity for estrogen due to its non-polar nature. A randomized clinical control trial by Gann et al. discovered that women on an isocaloric low-fat, high-fiber diet intervention had a 7.5% reduction in serum estradiol, compared to women following their normal diet (Gann et al., 2003). A meta-analysis of 10 intervention studies reported that a low-fat, high fiber diet lowered estrogen levels in premenopausal women (Wu, Pike, & Stram, 1999), and Tsuji et al. confirmed the effect of specifically saturated fat on increasing total and free estradiol levels (Tsuji et al., 2012). Decreased estrogen can lead to menstrual dysfunction and amenorrhea, and can also have

deleterious effects on bone remodeling. Because estrogen plays a large defensive role against oxidative stress in bone (Manolagas, 2010), as well as attenuating endocortical resorption of bone (Almeida et al., 2013), low estrogen levels, low saturated fat intake, and high fiber intake would amplify the impact of low estrogen on bone mineral density in amenorrheic athletes. The data support fiber's effect on estrogen in that total dietary fiber had a significant negative correlation with serum estradiol levels (r=-.4412,p=.0002), which persisted after controlling for BMI and duration of amenorrhea (**Figure 3)**.

Gibbs et al found that low BMI was a significant risk factor for low bone mineral density in exercising women (Gibbs et al., 2013). Previous studies have shown that dietary fiber is inversely associated with body weight and body fat (Slavin, 2005). In a study in rats, Islam et al. reported that consuming dietary fiber leads to marked metabolic alterations, including a reduction in adiposity and increase in lean body mass (Islam, Civitarese, Hesslink, & Gallaher, 2012). Additionally, they found that fiber leads to decreased plasma concentrations of leptin and resistin, two adipose derived hormones, and an increase in fatty acid oxidation markers and enzymes involved in mitochondrial biogenesis. Therefore, and increased intake of dietary fiber may lead to reduced adiposity and BMI, which presents an increased risk for low BMD.

A possible limitation of this study arises from the potential bias in food record recording. Even though thorough instructions on how to complete food records were given to each participant, there may be a discrepancy in nutritional knowledge between subjects which affects how they record components of their dietary intake. Because more participants were AA compared to EUM or NA, there may be confounding by indication

due to a selected sample. AA were more likely to complete and send back their food logs than EUM or NA. Additionally, the cross-sectional nature of this study prevents determination of direct causation of macro-and micro-nutrients on bone mineral density. Further prospective clinical research needs to be conducted to determine if the correlations between the nutrients and BMD are sustained.

Strengths from this study include the careful selection criteria for participants, which resulted in a representative sample of normal-weight amenorrheic and eumenorrheic athletes, and non-athletes. Also, supplement data was recorded along with food nutritional data, which gave an accurate estimate of vitamin, mineral, and fiber intake. Future studies should aim to determine the association of dietary fiber and saturated fat on bone mineral density while controlling for duration of amenorrhea, % body fat, total energy intake (kcal), and serum calcium levels. Percent body fat may be a more reliable indicator of energy status, because it is calculated by DXA, and does not rely on participant food records.

CONCLUSIONS

The food record data from groups of amenorrheic and eumenorrheic athletes and non-athletes reveal that athletes with menstrual dysfunction have significant differences in dietary intake which may have an effect on bone mineral density. Even though no differences in energy availability were observed between the groups, AA had a lower REE, which is indicative of a hypometabolic state. Because there was no difference in

overall caloric intake between groups, it is possible the high dietary fiber intake by AA reduces the metabolizable energy of food. Fiber and phytic acid's ability to alter the absorption of minerals involved in bone remodeling (i.e. magnesium, phosphorus, and calcium), and fiber's strong negative association with lumbar spine BMD suggest that it may exert direct and indirect mechanistic effects on bone metabolism. Further study of fiber's effect on the bioavailability of calcium, magnesium, phosphorus, and vitamin D is warranted. Additionally, it may be beneficial to study the effects of varying ratios of fiber to saturated fat on estrogen levels in a clinical study of amemorrheic athletes, who may already have compromised levels of circulating hormones. Ultimately, this study demonstrates that nutritional recommendations for the treatment of the Female Athlete Triad should include an analysis of the fiber content and fiber/fat ratio of the diet in addition to increased caloric intake and energy availability.

LIST OF JOURNAL ABBREVIATIONS

Am J Sports Med	The American Journal of Sports Medicine
Ann N Y Acad Sci	Annals of the New York Academy of Sciences
Arch Pediatr Adolesc Med	Archives of Pediatric and Adolescent Medicine
Arch Pediatr Adolesc Med	Archives of Pediatric and Adolescent Medicine
Br J Sports Med	British Journal of Sports Medicine
Endocr Rev	Endocrine Reviews
Fertil Steril	Fertility and Sterility
Int J Rheum Disease	International Journal of Rheumatic Diseases
J Am Coll Nutr	The Journal of the American College of Nutrition
J Appl Physiol	Journal of Applied Physiology
J Athl Train	Journal of Athletic Training
J Clin Endocrinol Metab	Journal of Clinical Endocrinology and Metabolism
J Clin Invest	Journal of Clinical Investgation
J Nutr	Journal of Nutrition
J Nutr	Journal of Nutrition
J Sports Sci	Journal of Sports Sciences
Med Sci Monit	Medical Science Monitor
Med Sci Sports Exerc	Medicine & Science in Sports & Exercise
N Engl J Med	New England Journal of Medicine
Orthop Clin North Am	Orthopedic Clinics of North America
Pediatr Clin North Am	Pediatric Clinics of North America

Phys Sportsmed	The Physician and Sports Medicine
Proc Nutr Soc	Proceedings of the Nutritional Society
Public Health Nutr	Public Health Nutrition
Sports Med	Sports Medicine

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CURRICULUM VITAE

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Education

University of Notre Dame, Class of 2013

- Major in Pre-Professional Studies and Psychology; Minor in Italian
- Cumulative GPA 3.4/4.0

Boston University Graduate Medical Sciences, Class of 2015

- MS in Nutrition and Metabolism

Clinical Experience

Patient Care Technician - May-August 2013
McLaren Northern Michigan – Petoskey, MI

- Performed basic patient care in the Intensive Care Unit
- Developed proficiency in glucose monitoring, catheter insertion, ECGs, and wound care
- Acted as a safety ambassador

Ambassador Volunteer - August 2012 – May 2013
Memorial Hospital – Mishawaka, IN

- Communicated with doctors and nurses about patients before and after surgery
- Offered comfort and direction to the families and friends of patients undergoing surgery

Intern, Orthopedics - June-August 2011
Azienda Ospedaliera Universitaria - Siena, Italy

- Assisted with post-surgery wound care
- Observed multiple orthopedic surgeries
- Assisted with cast construction and patient care in the orthopedics emergency room

Emergency Room Volunteer - August-December 2010
Saint Joseph Mercy Hospital - Mishawaka, IN

- Aided in patient transport and administered meals/water
- Assisted nurses in patient care

Research Experience

Graduate Student Intern - May 2014-Present
Neuroendocrine Unit – Massachusetts General Hospital

- Designed and facilitated a study on nutrition and bone metabolism in athletes
- Demonstrated proficiency in statistical analysis software

Research Assistant - January 2010-May 2013
Infant Studies Lab of Notre Dame – Notre Dame, IN

- Organized subject recruiting from the community
- Performed data analysis
- Initiated and developed a study on bilingual language development in Infants

Psychology Department Assistant - August 2012-May 2013
Attention and Memory Lab – Notre Dame, IN

- Conducted clinical lab studies
- Assisted in data analysis and interpretation

Certifications

- **Emergency Medical Technician,** State of Massachusetts - **July 2014-Present**