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A High Fiber and Vegetable Protein Diet is Associated with Low Lumbar Bone Mineral Density in Young Oligo-amenorrheic Athletes

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Abstract

Background—Associations of bone mineral density (BMD) with specific food components, including dietary fiber and isoflavones (that have a negative association with serum estrogen), are unclear and need to be determined, particularly in a population more likely to consume large amounts of these nutrients (such as young athletes).

Objective—To determine dietary intake of specific food components in oligo-amenorrheic athletes (OA) compared to eumenorrheic athletes (EA) and non-athletes (NA), and associations of the dietary intake of these nutrients with lumbar spine BMD.

Design and Subjects—This cross-sectional study evaluated 68 OA, 24 EA, and 26 NA 14–23 years old. Measurements included four-day food records (assessed using Nutrient Data System for Research software), a DXA scan evaluating lumbar spine BMD and body composition, and hormone levels. Multivariate analysis was used to estimate associations of nutrients with lumbar spine BMD.

Results—Compared with EA and NA, OA had higher intake of fiber, phytic acid, and vegetable protein (p<0.0001 for all). Intake of isoflavones, genistein and daidzein, was higher in OA than NA (p=0.003 and 0.0002 respectively). OA had lower consumption of energy from saturated fatty acids (%SFA) than NA (p=0.002). After controlling for confounders such as body weight, menstrual status (indicative of estrogen status), calcium intake, and serum vitamin D (known BMD determinants), lumbar spine BMD Z-scores were inversely associated with dietary fiber [β coefficient (β)= −0.30; p=0.01], vegetable protein (β = −0.28, p=0.02), phytic acid (β = −0.27, p=0.02), genistein (β = −0.25, p=0.01), and daidzein (β = −0.24, p=0.01), and positively with %SFA (β =0.32, p=0.0006)].

Conclusions—Compared to EA and NA, OA had a higher dietary intake of fiber, vegetable protein and phytic acid, which were inversely associated with lumbar spine BMD Z-scores. Further studies are needed to assess dietary recommendations for OA to optimize bone accrual.

Keywords

Dietary intake; diet; athletes; amenorrhea; bone density; adolescents

Introduction

Young women participating in sports that involve leanness, aesthetics, and endurance are at risk of developing the Female Athlete Triad (Triad), the interrelationship between low energy availability, menstrual dysfunction and low bone mineral density (BMD).[1] Oligo-amenorrheic athletes (OA) may be able to maintain a relatively stable weight, while having a lower energy intake by diverting available energy from non-life threatening physiologic processes, such as reproduction, towards those necessary for survival.[2–4] Oligo-amenorrhea is associated with low estrogen levels, increased bone resorption and low BMD.[5] Although weight-bearing activity has a positive impact on bone in regularly menstruating athletes, [6] this effect is lost in those with menstrual dysfunction.[7] Twenty to 50% of OA have low BMD and 10–13% have osteoporosis.[1,7] Our group and others have reported a higher prevalence of stress fractures in this population as well as greater bone loss at the lumbar spine in relation to other bone sites.[7–10]

Current treatment strategies for the Triad are focused on increasing energy intake, reducing physical activity, or both.[11] Adding a daily calorie supplement may help improve energy balance by providing additional calories and cause menstrual restoration.[12] However, the impact of such interventions on BMD remains unclear.[13] In addition, although BMD improves somewhat with weight gain and menstrual resumption, athletes may never completely normalize their BMD,[14] particularly when oligo-amenorrhea begins during the adolescent years of peak growth (11–14 years in girls with 90% of peak bone mass accrued

by 18 years).[15] It is thus possible that along with optimizing energy intake, it is essential to optimize intake of specific nutrients that impact bone.[11,16,17]

Optimizing calcium and vitamin D intake may have a favorable impact on bone mineralization,[18] however, certain other nutrients (such as an excess of dietary fiber) may be associated with unfavorable bone outcomes. Although diets rich in fiber are generally recommended for their beneficial health effects, such as weight regulation[19] and protection against chronic disease[20,21], a diet low in energy density and high in fiber has been linked to female endurance athletes with menstrual dysfunction.[22–24] In addition, certain athletes have been reported to favor a diet rich in vegetable protein, composed of whole grains, soy products, beans and legumes, and fruits and vegetables.[25] These diets are also high in dietary fiber, phytic and oxalic acid, and phytoestrogens, and low in saturated fat.[24,26–28] Dietary fiber can bind to and reduce absorption of phytoestrogens, [29] and its overconsumption may thereby negatively impact bone. In addition, phytates are known to bind minerals, protein and starch, reducing their digestion and absorption. [27] While the intake of these dietary components at recommended levels in healthy individuals may not impact bone, the overconsumption of these food components in OA in a state of chronic energy deficiency may be detrimental. Few studies have comprehensively examined dietary nutrient composition in athletes in relation to bone.

The purpose of this cross-sectional study was to determine dietary macro- and micronutrient composition in adolescent and young adult OA compared with eumenorrheic athletes (EA) and non-athletes (NA), and how group differences in nutrient intake may relate to lumbar spine BMD, the site most affected in OA and most likely to be impacted by onset or resumption of menses. [30–34] We hypothesized that specific patterns of nutrient intake in OA may have deleterious effects on bone.

Subjects and Methods

Participant Selection

118 female participants 14–23 years old were enrolled in this study at the Clinical Research Center of our institution between 2009 and 2014. Data being reported represent baseline data from an ongoing randomized controlled trial. Participants were recruited from local medical clinics, through contact with area coaches, advertising through social media, and postings around the Boston area. Sixty-eight athletes were OA, 24 athletes were EA, and 26 participants were NA. Inclusion criteria included a BMI between the 10th and 90th percentiles based on CDC growth charts,[35] and a bone age   14 years (98% of adult height is reached at a bone age of 14 years). Athletes were categorized as oligo-amenorrheic (OA) if they had absence of menses for   3 months within a period of oligomenorrhea (cycle length > 6 weeks) for   6 months, or absence of menarche at   16 years. Athletes and controls were categorized as eumenorrheic if they had   9 menses (cycle length 21–35 days) in the preceding year. Participants in both athlete groups were required to be engaged in   4 hours per week of aerobic weight-bearing training of the legs or   20 miles of running weekly for a period of   6 months in the previous year. Only endurance athletes engaged in weight-bearing activities of the legs were included in this study to minimize variation from different types of mechanical loading, and because such athletes, when oligo-amenorrheic,

are known to be at risk of low BMD.[10] Endurance athletes were primarily track and field and cross-country athletes and dancers. We excluded rowers, swimmers, cyclists, and gymnasts because of potential variability in weight-bearing activity. Non-athletes could not engage in >2 hours of weight-bearing activity per week, and could not be involved in team sports. Exclusion criteria for all groups included use of medications that affect bone metabolism, and conditions other than endurance training that cause amenorrhea. The study was approved by our Institutional Review Board.

Study Protocol

Consent (if the participant was   18 years) or assent and parental consent (if the participant was <18 years) were obtained at the screening visit. Eligibility was determined through a history and physical examination, self-report of menstrual status, and labs including a pregnancy test, complete blood count, and levels of thyroid stimulating hormone, follicle stimulating hormone, estradiol, calcium, phosphorus, and 25(OH) vitamin D (25(OH)D). Height was measured on a single wall-mounted stadiometer as the average of three measurements, and weight on an electronic scale. Body mass index (BMI) was calculated as the ratio of weight (in kg)/[height (in meters)]^2. An x-ray of the wrist and hand was taken to determine bone age (or maturity).[36] BMD, fat and lean mass were determined using dual-energy x-ray absorptiometry (DXA) (Hologic QDR-Discovery A, Apex v13.3; Hologic Inc, Waltham, Massachusetts).

To assess dietary intake, participants completed a 4-day food and supplement diary validated for use in young women (three weekdays and one weekend day). [37–39] Description of portion sizes and preparation methods were recorded and data analyzed by the Metabolism & Nutrition Research core of the Clinical Research Center of our institution using Nutrient Data System for Research software version 2008 (NDS-R) developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. [40] In a comparison of various methods of dietary intake assessment, 3-day food records had a higher correlation to estimates of food consumption from the reference method of a 9-day food record than did food frequency questionnaires. [40,41] The NDS-R report includes averages of total energy intake, and intake of specific macro- and micronutrients. Participants completed the Bouchard 3-day activity record for estimates of daily energy expenditure. Reliability assessment confirmed the reproducibility of the Bouchard record, with an interclass correlation of 0.96 for energy expenditure.[42] However, it includes both purposeful and non-purposeful exercise activity, and its validity for assessing specifically exercise energy expenditure in athletic populations has not yet been determined.[43] Hours per week of exercise activity for the study population was assessed independent of the Bouchard questionnaire at the time of the screening visit.

Statistical Methods

Data were analyzed using *JMP software* (v10; SAS Institute, Cary, NC). The study sample was based on baseline data from an ongoing RCT.[44] Group means were compared using ANOVA followed by the Tukey-Kramer test to adjust for multiple comparisons for normally distributed variables, or the Kruskal-Wallis test followed by the Steel Dwass test to adjust for multiple comparisons for non-parametric variables. Next, we examined

associations between nutrients specific to a diet rich in vegetable protein, namely dietary fiber, phytic and oxalic acid, and the soy isoflavones, but containing a low percentage of saturated fat (popular in some athletes), and lumbar spine BMD Z-scores. Correlations were analyzed by group (OA, EA, and NA) and all groups considered together and are reported as standardized (beta) coefficients (β). Multivariate analysis of selected nutrients was performed to assess if associations with lumbar spine BMD Z-scores persisted after controlling for potential confounders. These confounders included other variables that may impact BMD, namely duration of amenorrhea (indication of net estrogen status), low body weight (risk factor for low BMD), calcium intake (known determinant of BMD), and serum vitamin D (better indicator of vitamin D status than daily intake as factors such as season, clothing, skin color may impact levels).[45] Finally, we examined associations of dietary fiber and phytoestrogens with estradiol levels in a multivariate model controlling for duration of amenorrhea and fat mass (or body weight). We report standardized (β) coefficients (β) for associations between nutrients and lumbar spine BMD Z-scores before and after controlling for confounders.

Results

Participant Characteristics

EA were slightly younger than OA and NA (Table 1). Groups did not differ for height and weight. OA and EA had lower percentage of percent body fat than NA, while OA and NA had lower lean mass than EA ($p<0.05$). As expected, OA had lower lumbar spine BMD Z-scores than EA.

Macronutrient Analysis

OA, EA and NA did not differ for total energy intake or carbohydrate consumption (Table 2). However, OA consumed more vegetable and total protein (in grams) than EA and NA. Total fat intake (in grams) did not differ among groups, however percent of total calories derived from saturated fat was lowest in OA. OA had higher intake of total, soluble and insoluble fiber and pectin than EA (p 0.01 for all) and NA ($p<0.0001$ for all). No differences were observed between EA and NA for macronutrient intake.

Micronutrient Analysis

Calcium intake was higher in OA than NA, and phosphorus, magnesium and vitamin D intake higher in OA than EA or NA (Table 2). OA consumed more oxalic acid than NA, and more phytic acid than EA and NA. Intake of two phytoestrogens of the isoflavone class, genistein and daidzein, was higher in OA than NA. EA did not differ from NA.

Positive correlations were found for vegetable protein with total dietary fiber (r=0.87, $p<0.001$), phytic acid (r= 0.91, $p< 0.0001$), and genistein and daidzen (r=0.66, $p<0.0001$ for both), suggesting that a diet high in vegetable protein is also high in dietary fiber, phytic acid, and the soy isoflavones. Additionally, there were strong correlations for dietary fiber intake with intake of calcium (r= 0.51 $p=<0.001$), magnesium (r=0.82, $p=<0.001$) and phosphorus (r=0.74, $p=<0.001$), suggesting that girls who consumed large amounts of dietary fiber were also more likely to consume large amounts of these minerals.

Associations between Daily Nutrient Intake and Lumbar Spine BMD

For all groups combined, there was a negative correlation for lumbar spine BMD Z-scores with intake of vegetable protein, components of dietary fiber (total, soluble and insoluble), pectin, minerals (calcium, phosphorus and magnesium), phytic and oxalic acid, and the phytoestrogens (Table 3). A positive correlation was observed between lumbar spine BMD Z-scores and percent total energy derived from saturated fat. Similarly, OA had negative correlations for lumbar spine BMD Z-scores with intake of vegetable protein, total, soluble and insoluble fiber, phytic and oxalic acid, and the phytoestrogens, and positive correlations for lumbar spine BMD Z-scores with percent total energy derived from saturated fat.

When we analyzed associations for lumbar spine BMD Z-scores with mineral intake after controlling for fiber (as these were strongly correlated), the association was lost between lumbar spine BMD Z-scores and intake of calcium ($\beta = -0.49$; p=0.64), phosphorus ($\beta = 0.05$, p= 0.70), or magnesium ($\beta = -0.05$, p= 0.72), while total dietary fiber remained significantly associated with lumbar spine BMD Z-scores in each of these models [($\beta = -0.32$, p= 0.002); ($\beta = -0.38$, p= 0.004); ($\beta = -0.30$, p= 0.05), respectively] (data not shown in Table 3).

We next assessed whether associations for intake of selected nutrients with lumbar spine BMD Z-scores persisted after controlling for other potential confounders [duration of amenorrhea, body weight, calcium intake, and serum 25(OH) vitamin D] (see Statistical Methods), when the nutrients were added to this multivariate model one at a time. We included nutrients that differed across groups and were associated with lumbar spine BMD Z-scores, and certain nutrients related to a high vegetable protein, high fiber, and low saturated fat diet (such as phytic acid, oxalic acid, genistein, and daidzein). Vegetable protein, dietary fiber, phytic acid, genistein and daidzein remained negatively associated with lumbar spine BMD Z-scores in this multivariate model (Table 3). In addition, percent energy derived from saturated fat remained positively associated with lumbar spine BMD Z-scores ($\beta = 0.27$; p=0.01) after adding dietary fiber to the model.

Furthermore, after controlling separately for either total calorie intake, lean mass or body fat, associations persisted of lumbar spine BMD Z-scores with vegetable protein ($\beta = -0.43$, -0.32 and -0.27; p=0.004, 0.008 and 0.03 respectively), dietary fiber ($\beta = -0.38$, -0.33 and -0.29; p=0.003, 0.004, and 0.02), phytic acid ($\beta = -0.36$, -0.30 and -0.27; p= 0.004, 0.008 and 0.03), daidzein ($\beta = -0.25$, -0.26 and -0.24; p=0.01, 0.008 and 0.02), genistein ($\beta = -0.25$, -0.26 and -0.24; p=0.01, 0.008 and 0.02) and percent energy derived from saturated fatty acids ($\beta = 0.34$, 0.35 and 0.31; p= 0.0005, 0.0002 and 0.002).

For the OA group, percent energy derived from saturated fat remained positively associated with lumbar spine BMD Z-scores, while soluble fiber, daidzein, and genistein remained negatively associated with lumbar spine BMD Z-scores after controlling for possible confounders (duration of amenorrhea, body weight, calcium intake, and serum 25(OH) vitamin D) (Table 3).

Associations of Dietary Fiber and Phytoestrogens with Serum Estradiol

Because dietary fiber has previously been reported to bind to estradiol,[46] and genistein and daidzein exert estrogenic or anti-estrogenic effects (partial agonist-antagonist),[29] we examined associations of fiber and isoflavone intake with serum estradiol. Serum estradiol levels were correlated negatively with total, soluble and insoluble dietary fiber and pectin (β ≤ −0.33; p ≤ 0.008 for all), but not with genistein or daidzein (Table 4). On multivariate analysis, after controlling for duration of amenorrhea and fat mass, total, soluble and insoluble dietary fiber and pectin remained negatively correlated with serum estradiol (β ≤ −0.29; p ≤ 0.03 for all). Similarly, after controlling for duration of amenorrhea and body weight (instead of fat mass), total, soluble and insoluble dietary fiber and pectin remained negatively correlated with serum estradiol (β = −0.32, −0.37, −0.29, −0.28; p=0.02, 0.004, 0.03, 0.03 respectively).

Discussion

We demonstrate that although oligo-amenorrheic athletes (OA) had a higher intake of several macro- and micronutrients, a plant-based diet high in vegetable protein may be associated with lower lumbar spine bone mineral density in this population. We found differences between OA vs. EA and NA for intake of protein, fat, fiber, oxalate, phytate, and isoflavones. In contrast to expectations of positive associations of protein intake with BMD based on some [47] but not all [48] studies, we observed inverse associations of total and vegetable protein intake with lumbar spine BMD Z-scores. In addition, a higher intake of dietary fiber, phytates and oxalates was associated with lower BMD Z-scores, whereas a higher intake of saturated fat intake was positively associated with bone measures. These associations held after controlling for possible confounders. Because OA had higher vitamin D intake than EA and controls, and a higher calcium intake than controls, a deficiency in calcium and vitamin D intake cannot explain low BMD in OA.[9] Although nutrients consistent with a high vegetable protein diet are often considered to be beneficial to health[19], and USDA guidelines for females ages 19–30 suggest an intake for dietary fiber of 25g/day and an intake of protein and amino acids of 46 g/day, there are currently no USDA recommendations for phytic and oxalic acid, or upper level fiber consumption.[49] Therefore, we are concerned that consuming an excess of fiber, phytic and oxalic acid, isoflavones and vegetable protein, with an imbalance of other essential macro and micronutrients, may be detrimental to bone health in OA, who are in a state of chronic energy deficiency.

Because the BMI (per inclusion criteria) and percent body fat of the OA in our study were within a normal range, it may be necessary to develop a nutrition plan that not only increases overall energy intake, but takes into consideration specific dietary factors that impact bone, in order to reverse menstrual dysfunction and optimize BMD.[16] To maintain low body weight for aesthetic or performance purposes, active women may increase fruit and vegetable intake while decreasing dietary fat to reduce or maintain energy intake.[25] Such diets are typically rich in fiber. In addition, there has been a push by some sports nutritionists to encourage protein intake in athletes to increase energy intake and optimize bone health.[50,51] This may unfortunately be at the cost of fat intake, a known predictor of body fat mass.[52] Also, several macro- and micronutrients associated with these dietary

patterns (high fiber, high vegetable protein, and low saturated fat) may lead to unfavorable bone outcomes when consumed in excess. In this study, we first examined differences in nutrient intake in OA compared with EA and NA, and then assessed whether intake of a diet high in dietary fiber and vegetable protein, and low in saturated fat, was negatively associated with lumbar spine BMD Z-scores.

OA had a higher intake of dietary fiber than the other two groups, and dietary fiber remained negatively associated with lumbar spine BMD Z-scores even after controlling for body weight, menstrual status (measure of estrogen availability), calcium intake, and serum vitamin D levels (known positive determinants of BMD). Dietary fiber can affect dietary energy availability and digestibility of complex foods. It can interact with protein and fat, and decrease the metabolizable energy (ME) of a diet by affecting the digestibility of these components.[53] Because dietary fiber increases the bulk of intestinal contents and speeds up transit time in the gut, calcium and other minerals have less time to be absorbed.[23] Wolf et al. also found that fractional calcium absorption was inversely associated with dietary fiber intake, and women in the lowest tertile of the dietary fat/fiber ratio had 19% lower fractional calcium absorption than women in the highest tertile.[23] This may in part explain why OA exhibit lower BMD despite reporting a higher intake of nutrients such as calcium, magnesium, phosphorus and vitamin D. Further studies are needed to determine the change in average fractional calcium absorption due to a high fiber diet in our study population.

Similarly, phytic acid intake was higher in OA than in the other two groups and was associated negatively with lumbar spine BMD Z-scores. Importantly, phytic acid intake was also associated with fiber intake (r=0.88; p<0.001). Phytic acid has numerous anti-nutritional qualities because of its ability to bind to minerals, proteins, and starch, which can affect their solubility, functionality, digestion, and absorption. Minerals and nutrients most affected by phytic acid are calcium, magnesium, manganese, choline, zinc, sodium, iron, starch, and protein.[27] Foods rich in phytic acid, such as wheat bran, legumes, seeds, nuts, and soy isolates, may decrease the bioavailability and thus prevent the beneficial effects of nutrients such as calcium, magnesium and protein on bone.

Because dietary fiber remains a negative predictor and percent energy derived from saturated fat a positive predictor of lumbar spine BMD Z-scores after controlling for confounders, it is possible that the dietary fiber to fat ratio directly regulates bioavailability of certain macro- and micronutrients (already described) involved in bone metabolism. However, this ratio may also exert indirect effects through mechanisms affecting estrogen levels. A prospective study revealed that dietary fiber was inversely associated with LH, FSH, estradiol and progesterone levels, and positively with the risk of anovulation.[54] Dietary fiber can bind to sex hormones, specifically estrogen, due to its non-polar nature. This disrupts the enterohepatic circulation of estrogens by decreasing β-D glucuronidase activity and binding to estrogens in the intestine, which prevents estrogen reabsorption and facilitates excretion. A high fat/fiber ratio correlates negatively with estrogen excretion, and positively with serum estradiol.[46]

A randomized controlled trial demonstrated 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.[55] A meta-analysis of 10 intervention studies similarly reported that a low-fat, high-fiber diet lowers estrogen levels in premenopausal women,[56] while Tsuji et al. confirmed the association of specifically dietary saturated fat in increasing total and free estradiol levels.[57] Decreased estrogen can lead to menstrual dysfunction and amenorrhea, with deleterious effects on bone. Because estrogen plays a large defensive role against oxidative stress in bone,[58] as well as attenuating endocortical resorption of bone,[59] low estrogen levels along with low saturated fat intake and high fiber intake could amplify the impact of low estrogen on bone in OA. Our data are consistent in the association of fiber with estrogen levels in that total dietary fiber is associated negatively with serum estradiol even after controlling for fat mass and duration of amenorrhea.

Soy isoflavones (such as are genistein and daidzein) are phytoestrogens structurally similar to endogenous estrogens that can bind to the estrogen receptor and act as partial estrogen antagonists. During a dietary intervention with soy protein, midcycle surges of LH and FSH were suppressed.[60] Additionally, isoflavones have been shown to inhibit aromatase activity, a key enzyme in estrogen synthesis.[61] In an isoflavone feeding study in premenopausal women, soy isoflavone intake decreased levels of estrone, estradiol, estriol, and total estrogens.[62] Therefore, high isoflavone intake by OA may lead to lower lumbar spine BMD Z-scores through decreased estrogen levels or its partial estrogen antagonist effects. Genistein and daidzein are strongly associated with a diet rich in vegetable protein. Consistent with this, we observed strong associations between these isoflavones and vegetable protein intake in our study.

A limitation of this study is the bias inherent in food reporting. However, the food records we used in this study are well validated, [37–39][40,41], including in a study of adolescent girls with eating disorders as well as normal-weight adolescents.[44] Further, because more participants were OA compared to EA or NA, there is a higher likelihood of finding significant associations in the OA group than the other groups. Additionally, the cross-sectional nature of this study prevents determination of direct causation. Prospective studies are necessary to determine whether observed correlations between specific nutrient intake and lumbar spine BMD Z-scores are sustained in longitudinal sample. Conversely, strengths include the careful selection criteria for participants, which resulted in a well-differentiated sample of normal-weight oligo-amenorrheic and eumenorrheic weight-bearing endurance athletes, and non-athletes. Also, dietary supplement data were recorded along with food nutritional data, which resulted in a more accurate total estimate of vitamin, mineral, and fiber intake than food intake alone. Finally, we provide precise measurements of BMD assessed by DXA, and standardized by Z-scores.

CONCLUSIONS

Weight-bearing endurance athletes with menstrual dysfunction differ in dietary nutrient intake compared with normally menstruating athletes and non-athletes, which may affect their bone mineral density. We demonstrated that components of a diet high in vegetable protein and fiber and low in saturated fat are associated with lower bone mineral density at the lumbar spine, indicating possible direct and indirect deleterious effects on bone metabolism. These findings are preliminary and the influence of dietary fiber and phytic

acid on the absorption of minerals known to impact bone, and the impact of the fat/fiber ratio on estrogen levels warrants further study in the female athlete population. In healthy individuals, the intake of these foods at recommended levels likely does not significantly and negatively affect bone mass. In contrast, overconsumption of these food components, linked with chronic energy deficiency may be detrimental to bone.

Acknowledgments

References

1. Gibbs JC, Williams NI, De Souza MJ. Prevalence of individual and combined components of the female athlete triad. Med Sci Sports Exerc. 2013; 45(5):985–996. [PubMed: 23247706]

2. Nattiv A, Loucks AB, Manore MM, et al. American College of Sports Medicine position stand. The female athlete triad. Med Sci Sports Exerc. 2007; 39(10):1867–1882. [PubMed: 17909417]

3. Gibbs JC, Williams NI, Mallinson RJ, Reed JL, Rickard AD, De Souza MJ. Effect of high dietary restraint on energy availability and menstrual status. Med Sci Sports Exerc. 2013; 45(9):1790–1797. [PubMed: 23954993]

4. Thong FS, McLean C, Graham TE. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. J Appl Physiol (1985). 2000; 88(6):2037–2044. [PubMed: 10846016]

5. Nichols JF, Rauh MJ, Barrack MT, Barkai HS. Bone mineral density in female high school athletes: interactions of menstrual function and type of mechanical loading. Bone. 2007; 41(3):371–377. [PubMed: 17572167]

6. Lambrinoudaki I, Papadimitriou D. Pathophysiology of bone loss in the female athlete. Ann N Y Acad Sci. 2010; 1205:45–50. [PubMed: 20840252]

7. Ackerman KE, Cano Sokoloff N, DENMG, Clarke HM, Lee H, Misra M. Fractures in Relation to Menstrual Status and Bone Parameters in Young Athletes. Med Sci Sports Exerc. 2015; 47(8): 1577–1586. [PubMed: 25397605]

8. Rencken ML, Chesnut CH 3rd, Drinkwater BL. Bone density at multiple skeletal sites in amenorrheic athletes. JAMA. 1996; 276(3):238–240. [PubMed: 8667570]

9. Christo K, Prabhakaran R, Lamparello B, et al. Bone metabolism in adolescent athletes with amenorrhea, athletes with eumenorrhea, and control subjects. Pediatrics. 2008; 121(6):1127–1136. [PubMed: 18519482]

10. Barrack MT, Gibbs JC, De Souza MJ, et al. Higher incidence of bone stress injuries with increasing female athlete triad-related risk factors: a prospective multisite study of exercising girls and women. Am J Sports Med. 2014; 42(4):949–958. [PubMed: 24567250]

11. De Souza MJ, Nattiv A, Joy E, et al. 2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. Br J Sports Med. 2014; 48(4):289. [PubMed: 24463911]

12. Dueck CA, Matt KS, Manore MM, Skinner JS. Treatment of athletic amenorrhea with a diet and training intervention program. Int J Sport Nutr. 1996; 6(1):24–40. [PubMed: 8653102]

13. Cialdella-Kam L, Guebels CP, Maddalozzo GF, Manore MM. Dietary intervention restored menses in female athletes with exercise-associated menstrual dysfunction with limited impact on bone and muscle health. Nutrients. 2014; 6(8):3018–3039. [PubMed: 25090245]

14. Ducher G, Turner AI, Kukuljan S, et al. Obstacles in the optimization of bone health outcomes in the female athlete triad. Sports Med. 2011; 41(7):587–607. [PubMed: 21688870]

15. Bachrach LK. Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab. 2001; 12(1):22–28. [PubMed: 11137037]

16. Mountjoy M, Sundgot-Borgen J, Burke L, et al. The IOC consensus statement: beyond the Female Athlete Triad--Relative Energy Deficiency in Sport (RED-S). Br J Sports Med. 2014; 48(7):491–497. [PubMed: 24620037]

17. Beals, KA. Nutritional Needs of Female Athletes: From Research to Practice. Beals, KA., editor. Boca Raton, FL: CRC Press; 2013.

18. Anderson PH, Turner AG, Morris HA. Vitamin D actions to regulate calcium and skeletal homeostasis. Clin Biochem. 2012; 45(12):880–886. [PubMed: 22414785]

19. Slavin JL. Dietary fiber and body weight. Nutrition. 2005; 21(3):411–418. [PubMed: 15797686]

20. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. Metabolism. 2012; 61(8):1058–1066. [PubMed: 22401879]

21. Laughlin GA, Yen SS. Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. J Clin Endocrinol Metab. 1996; 81(12):4301–4309. [PubMed: 8954031]

22. Melin A, Tornberg AB, Skouby S, et al. Low-energy density and high fiber intake are dietary concerns in female endurance athletes. Scand J Med Sci Sports. 2015

23. Wolf RL, Cauley JA, Baker CE, et al. Factors associated with calcium absorption efficiency in pre- and perimenopausal women. Am J Clin Nutr. 2000; 72(2):466–471. [PubMed: 10919942]

24. Mangels AR. Bone nutrients for vegetarians. Am J Clin Nutr. 2014; 100(Supplement 1):469S–475S. [PubMed: 24898231]

25. Hill B, Williams N. 13. Dietary strategies contributing to low energy availability in exercising women: underlying physiology and impact on the menstrual cycle. Handbook of Diet and Nutrition in the Menstrual Cycle, Periconception and Fertility. 2014; (7):207.

26. Rodríguez R, Jimenez A, Fernández-Bolaños J, Guillén R, Heredia A. Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci Technol. 2006; 17(1):3–15.

27. Oatway L, Vasanthan T, Helm JH. Phytic acid. Food Rev Int. 2001; 17(4):419–431.

28. American Dietetic Association; Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. J Am Diet Assoc. 2003; 103(6):748–765. [PubMed: 12778049]

29. Anwar MJ, Radhakrishna KV, Sharma A, Vohora D. Raloxifene preserves phenytoin and sodium valproate induced bone loss by modulating serum estradiol and TGF-beta3 content in bone of female mice. Eur J Pharm Sci. 2014; 62:219–226. [PubMed: 24880111]

30. Drinkwater BL, Bruemner B, Chesnut CH 3rd. Menstrual history as a determinant of current bone density in young athletes. JAMA. 1990; 263(4):545–548. [PubMed: 2294327]

31. Ackerman KE, Sokoloff NC, De Nardo Maffazioli G, Clarke H, Lee H, Misra M. Fractures in Relation to Menstrual Status and Bone Parameters in Young Athletes. Med Sci Sports Exerc. 2014

32. De Souza MJ, West SL, Jamal SA, Hawker GA, Gundberg CM, Williams NI. The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. Bone. 2008; 43(1):140–148. [PubMed: 18486582]

33. Gremion G, Rizzoli R, Slosman D, Theintz G, Bonjour JP. Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. Med Sci Sports Exerc. 2001; 33(1):15–21. [PubMed: 11194101]

34. Winston AP, Alwazeer AE, Bankart MJ. Screening for osteoporosis in anorexia nervosa: prevalence and predictors of reduced bone mineral density. Int J Eat Disord. 2008; 41(3):284–287. [PubMed: 18176948]

35. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data. 2000; (314):1–27. [PubMed: 11183293]

36. Greulich, W.; Pyle, S. Stanford University Press. Radiographic Atlas of Skeletal Development of the Hand and Wrist. 2. Stanford: 1959.

37. Sawaya AL, Tucker K, Tsay R, et al. Evaluation of four methods for determining energy intake in young and older women: comparison with doubly labeled water measurements of total energy expenditure. Am J Clin Nutr. 1996; 63(4):491–499. [PubMed: 8599311]

38. Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M. Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. J Am Diet Assoc. 2009; 109(3):479–485. 485 e471–473. [PubMed: 19248866]

39. Chinnock A. Validation of an estimated food record. Public Health Nutr. 2006; 9(7):934–941. [PubMed: 17010260]

40. 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. [PubMed: 3171020]

41. Yang YJ, Kim MK, Hwang SH, Ahn Y, Shim JE, Kim DH. Relative validities of 3-day food records and the food frequency questionnaire. Nutr Res Pract. 2010; 4(2):142–148. [PubMed: 20461203]

42. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. Am J Clin Nutr. 1983; 37(3):461–467. [PubMed: 6829488]

43. Wickel EE, Welk GJ, Eisenmann JC. Concurrent validation of the Bouchard Diary with an accelerometry-based monitor. Med Sci Sports Exerc. 2006; 38(2):373–379. [PubMed: 16531909]

44. Misra M, Tsai P, Anderson EJ, et al. Nutrient intake in community-dwelling adolescent girls with anorexia nervosa and in healthy adolescents. Am J Clin Nutr. 2006; 84(4):698–706. [PubMed: 17023694]

45. Gordon CM, Goodman E, Emans SJ, et al. Physiologic regulators of bone turnover in young women with anorexia nervosa. J Pediatr. 2002; 141(1):64–70. [PubMed: 12091853]

46. Adlercreutz, H. Anticarcinogenesis and Radiation Protection. Vol. 2. Springer; 1991. Diet, breast cancer and sex hormone metabolism; p. 389-400.

47. Hirota T, Nara M, Ohguri M, Manago E, Hirota K. Effect of diet and lifestyle on bone mass in Asian young women. Am J Clin Nutr. 1992; 55(6):1168–1173. [PubMed: 1595590]

48. Ince BA, Anderson EJ, Neer RM. Lowering dietary protein to U.S. Recommended dietary allowance levels reduces urinary calcium excretion and bone resorption in young women. J Clin Endocrinol Metab. 2004; 89(8):3801–3807. [PubMed: 15292308]

49. US Department of Agriculture ARS, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference. Sep. 2015 Release 28. Version Current. Internet: http://www.ars.usda.gov/nea/bhnrc/ndl

50. Rodriguez NR, Di Marco NM, Langley S. American Dietetic A, Dietitians of C, American College of Sports M. American College of Sports Medicine position stand. Nutrition and athletic performance. Med Sci Sports Exerc. 2009; 41(3):709–731. [PubMed: 19225360]

51. Genaro PD, Pinheiro MD, Szejnfeld VL, Martini LA. Dietary Protein Intake in Elderly Women: Association With Muscle and Bone Mass. Nutr Clin Pract. 2014

52. Berteus Forslund H, Lindroos AK, Sjostrom L, Lissner L. Meal patterns and obesity in Swedish women-a simple instrument describing usual meal types, frequency and temporal distribution. Eur J Clin Nutr. 2002; 56(8):740–747. [PubMed: 12122550]

53. Baer DJ, Rumpler WV, Miles CW, Fahey GC Jr. Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. J Nutr. 1997; 127(4):579–586. [PubMed: 9109608]

54. Gaskins AJ, Mumford SL, Zhang C, et al. Effect of daily fiber intake on reproductive function: the BioCycle Study. Am J Clin Nutr. 2009; 90(4):1061–1069. [PubMed: 19692496]

55. Gann PH, Chatterton RT, Gapstur SM, et al. The effects of a low-fat/high-fiber diet on sex hormone levels and menstrual cycling in premenopausal women: a 12-month randomized trial (the diet and hormone study). Cancer. 2003; 98(9):1870–1879. [PubMed: 14584069]

56. Wu AH, Pike MC, Stram DO. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J Natl Cancer Inst. 1999; 91(6):529–534. [PubMed: 10088623]

57. Tsuji M, Tamai Y, Wada K, et al. Associations of intakes of fat, dietary fiber, soy isoflavones, and alcohol with levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Causes Control. 2012; 23(5):683–689. [PubMed: 22399233]

58. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010; 31(3):266–300. [PubMed: 20051526]

59. Almeida M, Iyer S, Martin-Millan M, et al. Estrogen receptor-alpha signaling in osteoblast progenitors stimulates cortical bone accrual. J Clin Invest. 2013; 123(1):394–404. [PubMed: 23221342]

60. Cassidy A, Bingham S, Setchell KD. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr. 1994; 60(3):333–340. [PubMed: 8074062]

61. Campbell DR, Kurzer MS. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. J Steroid Biochem Mol Biol. 1993; 46(3):381–388. [PubMed: 9831487]

62. Xu X, Duncan AM, Merz BE, Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. Cancer Epidemiol Biomarkers Prev. 1998; 7(12):1101–1108. [PubMed: 9865428]

Table 1

Clinical Characteristics and Body Composition in Oligo-amenorrheic Athletes (OA), Eumenorrheic Athletes (EA) and Non-athletes (NA)

	OA (n=68) Mean±SD	EA (n=24) Mean ± SD	NA (n=26) Mean ± SD	ANOVA p-value
Age (years)	19.65 ± 2.58	18.21 ± 2.88	19.64 ± 2.18	**0.04**[A]
Height (cm)	165.1 ± 6.4	165.3 ± 7.7	161.7 ± 7.0	0.08
Weight (kg)	57.1 ± 8.7	58.9 ± 7.3	57.2 ± 8.2	0.17
BMI (kg/m²)	20.9 ± 2.4	22.2 ± 2.6	21.8 ± 2.5	**0.04**[A]
REE (kcal/kg FFM)	29.83 ± 5.58	30.79 ± 5.38	31.16 ± 4.76	0.51
Total Lean Mass (kg)	42.4 ±5.5	45.6 ±7.4	39.5 ± 4.7	**0.002**[A,C]
% Body Fat	23.3 ±4.8	23.6±4.1	28.1 ± 6.2	**0.002**[B,C]
Lumbar BMD Z-Scores	−0.66 ±1.21	0.03± 0.95	−0.39 ± 1.03	**0.04**[A]
Serum 25 (OH) vitamin D level (ng/ml)	37.05±11.88	30.67±14.36	21.78±7.21	**<0.001**[A,C]

[A] p<0.05 for OA vs. EA;

[B] p<0.05 for OA vs. NA;

[C] p<0.05 for OA vs. NA

OA: oligo-amenorrheic athletes; EA: eumenorrheic athletes; NA: non-athletes

Data presented as mean±SD. Significant p-values are bolded

ANOVA used for 3 groups comparisons followed by Tukey-Kramer test when ANOVA was significant

Table 2

Daily Macronutrient and Micronutrient Intake in Oligo-amenorrheic Athletes (OA), Eumenorrheic Athletes (EA) and Non-athletes (NA)

	OA (n=68) Mean±SD	EA (n=24) Mean±SD	NA (n=26) Mean±SD	ANOVA p-value
Macronutrient Intake				
Total caloric intake (kcal)	2206 ± 717	1918 ± 626	1874 ± 426	0.13[*]
Carbohydrates (g)	296.5 ± 95.6	266.9 ± 91.3	249.4 ± 63.8	0.1
Protein (g)	96.5 ± 37.4	76.4 ± 25.3	71.9 ±19.1	**0.001**[A,B]
Animal protein (g)	50.5 ± 29.0	44.5 ± 20.9	45.1 ± 15.6	0.76
Vegetable protein (g)	45.6 ± 19.2	31.9 ± 12.9	26.8 ± 8.8	**<0.0001**[*A,B]
Fat (g)	77.7 ± 35.6	65.5 ± 24.4	66.7 ± 21.6	0.34
% of total energy from saturated fat	8.6 ± 2.6	9.8 ± 2.2	10.6 ± 2.6	**0.002**[B]
% of total energy from mono-unsaturated fat	11.8±3.4	10.8±1.7	11.3±2.7	0.37
% of total energy from poly-unsaturated fat	7.3±2.4	6.8±1.6	6.4±1.8	0.15
Dietary fiber (g)	34.8 ± 15.3	22.5 ± 11.7	17.3 ± 6.6	**<0.0001**[*A,B]
Insoluble fiber (g)	25.4 ± 12.3	15.8 ± 9.1	11.4 ± 5.6	**<0.0001**[A,B]
Soluble fiber (g)	8.8 ± 3.5	6.5 ± 2.8	4.8 ± 1.8	**<0.0001**[A,B]
Pectin (mg)	4.93±2.62	3.01±1.49	2.27±0.92	**<0.0001**[A,B]
Micronutrient Intake				
Calcium (mg)	1595 ± 781	1218 ± 709	943 ± 394	**0.0002**[B]
Phosphorous (mg)	1597 ± 567	1243 ± 463	1149 ± 336	**0.0002**[A,B]
Magnesium (mg)	499 ± 220	314 ± 122	273 ± 86	**<0.0001**[A,B]
Vitamin D (mcg)	16.6 ± 16.7	8.4 ± 7.2	7.5 ± 7.2	**0.004**[A,B]
Phytic acid (mg)	1276 ± 621	621 ± 384	700 ± 370	**<0.0001**[*A,B]
Oxalic acid (mg)	541 ± 539	336 ± 263	247 ± 288	**<0.0001**[*B]
Daidzein (mg)	5.85 ± 8.02	1.97 ± 4.82	0.99 ± 2.89	**0.0002**[B]
Genistein (mg)	7.41 ± 10.09	2.72 ± 6.65	1.41 ± 4.22	**0.003**[B]

[A] p<0.05 for OA vs. EA;

[B] p<0.05 for OA vs. NA;

[C] p<0.05 for EA vs. NA

OA: oligo-amenorrheic athletes; EA: eumenorrheic athletes; NA: non-athletes

Data presented as mean±SD. Significant p-values are bolded

ANOVA used for 3 groups comparisons followed by Tukey-Kramer test when ANOVA was significant

[*] Values not normally distributed were compared by Kruskal Wallis Test, followed by Steel Dwass test.

Table 3

Associations of specific nutrients with lumbar spine BMD Z-scores in all groups considered together (oligo-amenorrheic athletes, eumenorrheic athletes and non-athletes), and in oligoamenorrheic athletes alone

| Daily nutrient intake | Oligo-amenorrheic Athletes, Eumenorrheic Athletes and Non-Athletes | | | | | | Oligo-amenorrheic Athletes Alone | | | | | |
| | Unadjusted | | | Adjusted | | | Unadjusted | | | Adjusted | | |
	β	R^2	p	β**	R^2	p	β	R^2	p	β**	R^2	p
Total protein	−0.23	0.05	0.01	−0.13	0.01	0.26	−0.16	0.03	0.20	−0.10	0.007	0.50
Vegetable protein	−0.34	0.12	0.0002	−0.28	0.04	0.02	−0.32	0.10	0.009	−0.29	0.05	0.06
Animal protein	−0.06	0.004	0.50	−0.0008	0.0	0.99	0.005	0.0	0.97	0.03	0.01	0.82
% Total energy from saturated fat	0.32	0.10	0.0004	0.32	0.10	0.0006	0.33	0.11	0.006	0.37	0.13	0.002
Total dietary fiber	−0.35	0.11	0.0001	−0.30	0.06	0.01	−0.30	0.09	0.01	−0.27	0.05	0.06
Soluble fiber	−0.36	0.13	<0.0001	−0.34	0.08	0.002	−0.35	0.12	0.003	−0.35	0.10	0.01
Insoluble fiber	−0.34	0.11	0.0002	−0.27	0.05	0.02	−0.29	0.08	0.02	−0.24	0.04	0.10
Pectin	−0.27	0.07	0.003	−0.18	0.02	0.10	0.22	0.05	0.07	−0.16	0.02	0.23
Calcium	−0.21	0.04	0.02	−0.15	0.02	0.13	−0.14	0.02	0.25	−0.09	0.006	0.50
Phosphorus	−0.23	0.06	0.01	−0.11	0.006	0.41	−0.12	0.01	0.33	−0.01	0.0	0.95
Magnesium	−0.30	0.09	0.0009	−0.29	0.04	0.04	−0.22	0.05	0.07	−0.28	0.04	0.12
Vitamin D	−0.01	0.0	0.91	0.13	0.01	0.27	−0.01	0.0	0.91	0.17	0.02	0.27
Phytic acid	−0.35	0.12	0.0001	−0.27	0.05	0.02	−0.29	0.08	0.02	−0.22	0.04	0.12
Oxalic acid	−0.21	0.04	0.02	−0.16	0.01	0.12	−0.24	0.06	0.05	−0.18	0.03	0.19
Daidzein	−0.32	0.10	0.0005	−0.24	0.05	0.01	−0.34	0.11	0.005	−0.27	0.07	0.03
Genistein	−0.33	0.11	0.0003	−0.25	0.05	0.01	−0.36	0.13	0.003	−0.28	0.07	0.03
Serum 25(OH) vitamin D	−0.07	0.005	0.48	0.02	−0.06	0.84	−0.02	0.0	0.89	0.07	−0.04	0.61

β : β (or standardized) estimate; β **: β (or standardized) estimate after controlling for duration of amenorrhea, body weight, calcium intake, and serum vitamin D

R^2: R-Squared values

Table 4

Correlations of nutrients associated with a high vegetable protein diet with serum estradiol levels for all groups taken together (oligo-amenorrheic athletes, eumenorrheic athletes and non-athletes)

	Unadjusted			Adjusted		
	β	R^2	p	β **	R^2	p
Total dietary fiber	−0.36	0.13	0.004	−0.32	0.09	0.02
Soluble fiber	−0.41	0.17	0.0007	−0.40	0.13	0.004
Insoluble fiber	−0.33	0.11	0.008	−0.29	0.07	0.03
Pectin	−0.33	0.11	0.007	−0.29	0.07	0.03
Genistein	−0.08	0.006	0.53	0.05	0.001	0.77
Daidzein	−0.11	0.01	0.40	−0.004	0.0	0.98

β: β (or standardized) coefficient

β **: β (or standardized) coefficient after controlling for duration of amenorrhea and body fat mass

R^2: R-Squared values