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EXPLORING DIETARY INTAKES OF MICRONUTRIENTS INVOLVED IN BONE REMODELING IN RELATION TO BONE MINERAL DENSITY AND PHYSICAL ACTIVITY LEVELS: AN EXAMINATION OF PRE-MENARCHEAL FEMALE GYMNASTS AND NON-GYMNASTS.

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ABSTRACT

Objective: The purpose of this study was to explore the relationships between dietary intake of vitamin K, vitamin A, vitamin D, fiber, and bone mineral density in a sample of pre-menarcheal female gymnasts and non-gymnasts in the Syracuse-area. The goal was to observe whether higher intakes of these vitamins were associated with higher bone mineral density as measured by dual-energy X-ray absorptiometry (DXA), and to potentially examine the impact of activity level on these relationships.

Methods: A cross-sectional design was used to examine a subset of data from a larger on-going longitudinal study on bone growth and physical activity in young females. The original study was designed to compare activity, and bone density data between gymnast and non-gymnasts. The data used in this analysis were collected from January 2009 to September 2012 from 52 pre-menarcheal female gymnasts and non-gymnasts between the ages of 9-11. Participants were asked to complete Harvard's Youth/Adolescent Questionnaire biannually to quantify nutrient intake. Annual bone density measurements were taken of the lumbar spine and total body using dual-energy X-ray absorptiometry (DXA). Participants also were asked to self-report physical activity semi-annually during an interview session in which they described the type of activity and hours per week of participation. Tanner breast and pubic stages were self-assessed by subjects at each interview session. Standing height, sitting height, and weight measures were collected at the interview sessions as well.

Participants: The participants were 52 pre-menarcheal female gymnasts and non-gymnasts between the ages of 9-11. The selection criteria for this analysis was limited to participants who

self-reported as Tanner Stage I (Tanner breast I and Tanner pubic I) at the time of the annual DXA scan. This criterion minimizes the potentially confounding effects of estrogen on bone density and mineral concentration. Non-Caucasian subjects were not included in this analysis as the original cohort of subjects were predominantly Caucasian and the sample size for the current study was too small to account for racial variation. A factor analysis was performed to decrease the dimensionality of the full data set. A principle component analysis was used to observe groupings of dietary variables and to better understand over all dietary patterns. Pearson correlations, and simple linear regressions were used to identify associations between specific micronutrients and minerals and bone mineral density. Multiple regression analyses and ANOVA (general linear model, GLM) were used to further examine the relationships relationship between bone mineral density and the intake of vitamin A, vitamin D, biologically active vitamin K, and the ratio of vitamin A:D intake while adjusting for physical activity, weight, and age of participants. Statistical significance for all tests was set at the $\alpha=0.05$ level.

Results: Inverse relationships were found between bone mineral density and dietary intake of vitamin A ($p=0.018$), beta-carotene ($p=0.008$), fiber ($p=0.029$), and carotene ($p=0.007$). Bone mineral density increased with a combination of increased vitamin K1 intake and increased body mass ($p=0.0006$) and vitamin A and increased body mass ($p=0.0001$). A complex relationship appears to exist between bone mineral density and the combination of vitamin A and vitamin D intakes. The lowest bone mineral densities were observed in the highest quartile of vitamin A intake; this quartile was well above the tolerable upper limit for the age range of the focal population age range. The lowest bone densities were also associated with the highest levels of vitamin D, which was an unexpected result. However, this quartile of vitamin D intake was also

above daily recommend intake levels for the focal population. Although not statistically significant, bone mineral density was lowest when vitamin A was either very high (4th quartile of vitamin A intake, mean ± stdev = 17,317.34 ±4358.86 IU) or very low (1st quartile of vitamin A intake, mean ± stdev = 5243.01 ±1230.68 IU) with respect to vitamin D intake. Intermediate levels of the ratio of the vitamin A intake with reference to vitamin D intake produced the highest bone densities.

Conclusions: Higher intakes of fiber, vitamin A, carotene, and beta-carotene appear to be associated with lower measures of bone mineral density in this study population. Increased difference in the intake of vitamin A with respect to vitamin D appears to be associated with lower measures of bone mineral density, even with intake levels of vitamin D above the EAR and RDA suggested for this age group. This relationship may result from excess preformed vitamin A ingestion leading to increased retinoic acid in the body. Excess retinoic acid has the potential to impair vitamin D's ability to carry out its own genomic functions. These relationships warrant further exploration in order to improve the understanding of the genomic impact of vitamin A's impact on bone metabolism in the preadolescent life stages, as well as to aid in the prevention of negative effects on bone mineral density during this time period.

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Part I: Literature Review and Proposed Study Design

Introduction

The purpose of this study is to explore the relationships between dietary intake of foods rich in vitamin K1, calcium, vitamin A, vitamin D and bone density in young female gymnasts and a comparison group of active non-gymnasts. We would like to determine if higher levels of vitamin intake in young females are associated with higher levels of bone density.

Physical Activity, Bone Growth, and Remodeling

Skeletal growth in adolescence is characterized by the synthesis of new bone through endochondral ossification as well as the modeling and remodeling of existing bone.[1] It has been well-documented that significant bone adaptations and remodeling occur following the introduction of high-intensity weight-bearing activities during childhood, and growing bone possesses a greater capability to adapt to mechanical loading.[2] It has also been postulated that the skeletal benefits that are acquired during this period of growth are able to be retained to some extent, potentially aiding in the prevention of fractures later in life.[3] Therefore, we are interested in further exploring the associations between physical activity levels and changes in bone parameters over time in pre-pubescent females. In particular, we are interested in females participating in artistic gymnastics; there is a vast body of work that indicates that participation in high-impact activities such as artistic gymnastics significantly increases skeletal growth in pre-pubertal girls. There is also evidence that these young gymnasts tend to display higher levels of bone mineral content (BMC) and bone mineral density (BMD) than their non-gymnast peers, potentially allowing for a greater protective effect against fractures later in life.[4] This evidence is

consistent with the knowledge that exercise involving heavy impact and frequent mechanical loading is able to trigger the bone remodeling process, leading to changes in bone structure and increased BMC and BMD.[5]

Bone modeling and remodeling are processes that are central to the development and maintenance of the skeletal system. Remodeling involves the constant removal of packets of old bone and then immediately replacing this area with new bone through mineralization of the matrix, therefore preventing bone microdamage from accumulating and causing significant damage.[6] Osteoclasts and osteoblasts are at the core of this process, with the former responsible for the removal of old bone and the latter involved in the formation of new bone. Osteoclasts are the only known cells capable of resorbing bone. They are derived from mononuclear monocyte-macrophage precursor cells, and their formation is dependent on receptor activation of NF-κβ ligand (RANKL) and macrophage CSF (M-CSF) cytokine.[6] Activated osteoclasts carry out their required duty by binding to bone matrix and releasing acidified vesicles to the bone resorption surface that break down one particular region, with the surrounding bone surface protected through a simultaneous mechanism that seals off the resorption area.

In contrast, osteoblast activity involves the synthesis and calcification of bone matrix to create new bone.[7] These cells are derived from osteoprogenitor cells that arise from pluripotent stem cells, and are responsible for the deposition of bone matrix in intramembraneous and endochondral bone formation.[8] The third major cell type involved in bone remodeling is the osteocyte. These cells are former osteoblasts that become trapped during bone deposition and thus become part of the mineralized bone matrix.[9] In recent years, an increasing body of evidence has implicated osteocytes as being primarily responsible for bone's adaptation to mechanical forces placed upon it, but the molecular mechanisms have yet to be elucidated.

Additionally, osteocytes have been found to be regulators of osteoblast and osteoclast production and function, giving them a critical role in the bone remodeling cycle.

A multitude of dietary components also come in to play when dealing with bone mineral density, bone mineral concentration, and the process of bone remodeling; calcium, vitamin D, vitamin A, vitamin K, and the omega-3 fatty acids have been found to be of particular significance. In brief, vitamin D has been found to play an important role in stimulating osteoblastogenesis in mesenchymal stem cells as well as affecting bone mineralization through its function in intestinal calcium absorption and bone mineral accretion.[10] Calcium's relationship to bone health has been extensively researched, and increased calcium intake has been strongly associated with increased bone accrual, as well as decreased parathyroid hormone (PTH) levels and decreased bone resorption.[11]

Vitamin A's role in bone health is less clear, but is known to be involved due to nuclear receptors for retinoic acid in osteoblasts and osteoclasts.[12] Some studies have proposed that increasing vitamin A intake may decrease BMD and promote fractures, while others have not shown any increase in bone loss or fracture risk, and still others have suggested that increased vitamin A intake may be protective from bone loss.[13] Therefore, extensive research is still required to create a clearer picture of where vitamin A fits in to the bone remodeling process as well as to determine what forms of vitamin A have the greatest impact. Omega-3 fatty acids have been more recently linked to the bone remodeling process, as recent human studies have reported long-chain polyunsaturated fatty acids (LCPUFAs) increasing bone formation, leading to a positive effect on peak bone mass and reducing bone loss in adolescents.[14] The exact mechanism of how these LCPUFAs can act on bone remodeling is still being investigated, but is postulated to be related to their role in the release of the receptor-activated nuclear factor-κβ ligand

(RANKL), which is involved in the differentiation of osteoclasts in osteoclast precursors.[15] Finally, vitamin K has a well-established connection to bone health as it is a necessary cofactor for the gamma-carboxylation of osteocalcin, a noncollagenous bone protein produced by osteoblasts that is used as a biomarker for bone formation.[12] Additionally, epidemiological studies have shown an association between low vitamin K intake and lower BMD, although randomized controlled trials have shown conflicting results as well as have suggested differences in the function of vitamin K1 (phylloquinone) and vitamin K2 (menaquinones) in regards to bone health.

Mineral Metabolism and Calcium

Calcium homeostasis plays a key role in multiple skeletal and cellular processes in the human body, and therefore is tightly controlled. Dietary calcium is absorbed across the intestinal epithelial brush border primarily through the calcium channel TRPV6, while a smaller amount is able to be absorbed through paracellular diffusion. Once inside the cell, calcium is bound to transport protein calbindin for transportation to the endoplasmic reticulum and then to the basolateral membrane of the cell.[16] Calcium is then able to enter the bloodstream through use of a Ca^{2+}-ATPase pump, allowing it to circulate throughout the body.[17] Serum calcium levels are regulated to remain between 8.5 to 10.5 mg/dL by an endocrine system involving multiple feedback systems and use of vitamin D metabolites such as calcitriol and parathyroid hormone (PTH). If levels are too low, the parathyroid gland is signaled to secrete more PTH, which is then able to signal for increased bone resorption to increase serum calcium levels. PTH also acts to stimulate the kidneys to produce more calcitriol and decrease urinary calcium excretion. The calcitriol produced by the kidneys signals to the epithelial cells of the small intestine to increase

calcium absorption, further leading to increased serum calcium levels.[17] A feedback mechanism is then employed; as serum calcium levels increase to the normal range, PTH secretion decreases leading to decreased calcitriol production, slowed bone resorption, and the intestinal cells resume normal calcium absorption. If serum calcium levels are too high, the parathyroid gland is signaled to secrete less PTH. This leads to a decrease in calcitriol production and increased calcium excretion by the kidneys. The thyroid gland is also involved, as its "c" cells are signaled to release the peptide hormone calcitonin to halt bone resorption through inhibiting the activity of the osteoclast cells that release calcium from the bone.[17] A feedback mechanism is again employed to halt these responses as serum calcium levels decrease in order to maintain the narrow normal serum calcium range. The following diagram shows the endocrine regulation pathway in the case of hypocalcemia.

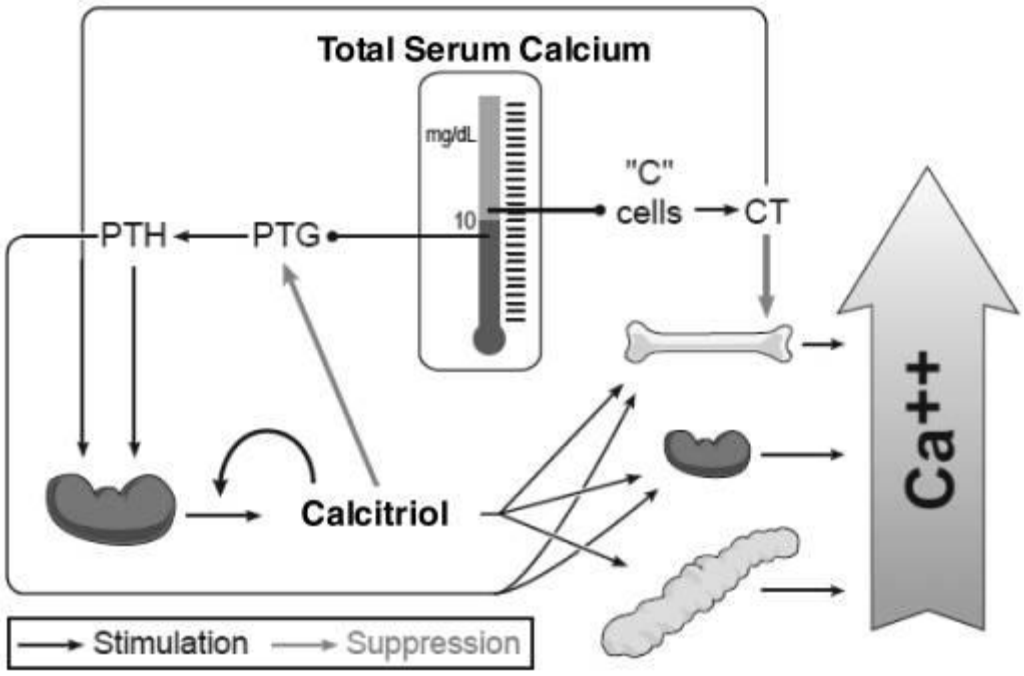


Figure 1: Endocrine regulation of serum calcium levels showing the actions of PTH and calcitriol on their respective organ targets. Note: PTG=Parathyroid gland; CT= Calcitonin. From Ross *et al*., 2011.[16]

The Role of Vitamin K in Mineral Metabolism

The group of K vitamins was first discovered by the Danish biochemist Henrik Dam in 1929 while conducting research on the use of fat-free diets in chicks. Dam observed that the chicks consuming the fat-free diet developed hemorrhages under the skin and muscle that could not be cured through use of vitamin C, A, or D supplementation, and he and his group determined that the delayed blood coagulation was the result of an absence of prothrombin activity in the plasma.[18] This led to the subsequent discovery of the fat-soluble vitamin K structure and the first hint at its role in the coagulation cascade. All compounds with vitamin K activity share a common 2-methyl-1,4-napthoquinone ring and are differentiated by their structure at the 3-position. Figure 2 below depicts the structures of vitamin K1, two forms of vitamin K2, and the synthetic vitamin K3. The most common form of vitamin K is vitamin K1, or phylloquinone, which has a phytyl substituent at the 3-position, and is most commonly found in green, leafy vegetables. Vitamin K2 consists of a group of vitamin K structures with a varying number of isoprenyl side chain units, known as menaquinones. The most common menaquinones are MK-4 and MK-7, which are primarily obtained in the diet through low amounts found in animal products such as meat, eggs, liver, and cheeses, as well as much higher amounts present in fermented foods.[19]

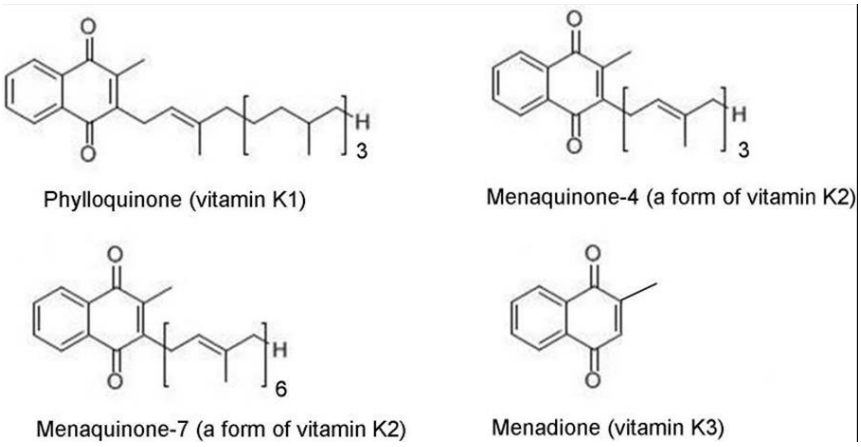


Figure 2: **Structures of phylloquinone, menaquinone-4, menaquinone-7, and menadione. From Shea *et al.*, 2016**.[20]

Forty years after Dam's group determined its existence, the mechanism of action of vitamin K was elucidated following the discovery of γ-carboxyglutamic acid (Gla) in 1974, which explained the prolonged prothrombin time observed in vitamin K deficiencies and led to the discovery of more vitamin K-dependent proteins (VKDPs). The currently known VKDPs include blood coagulation proteins such as prothrombin, factors VII, IX, X, protein C, protein S, and protein Z, and bone Gla proteins osteocalcin, MGP, and protein S. The blood coagulation VKDPs contain 10-12 Gla residues that enable Ca^{2+}-mediated binding of the proteins to negatively charged phospholipid surfaces of platelets and endothelial cells at a site of injury in vascular tissue, subsequently allowing the factor proteins to carry out their role in the blood clotting cascade.[18] This is traditionally the best known role of vitamin K; a cofactor in the carboxylation of glutamic acid residues to γ -carboxyglutamic acid in precursor proteins, allowing for binding of Ca^{2+} and subsequent participation in the clotting cascade.[21] However, this group of vitamins is becoming increasingly known for the essential role played during γ-carboxylation of the VKDP osteocalcin found in osteoblasts. Carboxylated osteocalcin binds to hydroxyapatite in mineralized tissues, creating a link between vitamin K and bone health.[22]

There is a substantial body of research that has examined relationships between vitamin K1 and bone health in different populations; in particular, osteocalcin has been frequently examined as a link between vitamin K1 and bone metabolism. A large portion of this research consists of population-based studies examining phylloquinone intake and bone mineral density (BMD), but results of these studies have been inconsistent. One of the first of such studies that explored this relationship was a prospective cohort, the Nurses' Health Study, which was conducted over a ten-year period and examined the effect of daily vitamin K intake on bone fragility indices. Data was obtained from three semi-quantitative food frequency questionnaires (FFQ) from 72,327 women, over a period of ten years; phylloquinone content of the foods on the FFQ was obtained from the USDA, and women were asked to report all hip fractures.[23]

Feskanich et al reported that food items contributing most to the women's dietary phylloquinone intake were iceberg lettuce, cooked spinach, cabbage, raw spinach, romaine, Brussels sprouts, and kale, and only 1-4% of participants reported receiving greater than 10µg/d of phylloquinones from supplementation. The risk of hip fracture was found to be inversely associated with the amount of lettuce consumed, when comparing data from those who reported eating one or more servings per day to those who reported eating one or fewer servings of lettuce per week. Additionally, it was found that women in quintiles 2-5 of phylloquinone intake had significantly lower age-adjusted relative risk of hip fracture than women in the lowest quintile, and the risk estimates did not change when other osteoporosis risk factors, such as vitamin D and calcium intake, were added to the model.[23] The authors concluded that low intakes of vitamin K may increase the risk for hip fracture in women.

Similar results were found upon examination of a Framingham Heart Study cohort of elderly men and women with a mean age of 75 years old, there was no significant association

found between phylloquinone intake and BMD; lower phylloquinone intake was found to be associated with an increased risk for hip fracture, but this also may indicate poorer overall nutritional status as phylloquinones are primarily found in green, leafy vegetables.[24] In the same study looking at a younger cohort with a mean age of 59, phylloquinone intake was found to be positively associated with BMD when examined cross-sectionally in women, but the same effect was not found in men. However, when this cohort was examined using a prospective analysis, there was no significant association between phylloquinone intake and BMD change over 5 years.[25]

There has been some examination of vitamin K1 intake and markers of bone formation in study samples more similar to ours in age and life stage, as Kalkwarf et al (2004) looked at vitamin K status and BMC in a study of 245 healthy girls aged 3-19 over a period of four years.[26] Phylloquinone intake was not found to be consistently associated with BMC or other bone turnover markers, but higher levels of plasma phylloquinone and lower percentage of undercarboxylated osteocalcin were found to be associated with lower bone resorption and formation. Additionally, plasma phylloquinone was found to have a statistically significant inverse relationship with osteocalcin concentrations. The authors of this study concluded that better vitamin K status was associated with decreased bone turnover in this study sample. However, they also acknowledged the need for randomized controlled trials examining the effects of phylloquinone supplementation on bone turnover in this age group to provide more conclusive evidence of vitamin K's suggested benefits to bone.

Similarly, a study by O'Connor et al (2007) examined the relationship between serum percentage of undercarboxylated osteocalcin (ucOC) and BMC in a study sample of 223 healthy girls aged 11-12 years.[27] Serum percentage ucOC was not found to be associated with markers of

bone resorption, but was found to have a significant inverse relationship with BMC of the total body as well as lumbar spine after adjusting for potential cofounders such as vitamin D status. The authors thus concluded that better vitamin K status was associated with higher BMC, but not bone turnover, in this population. Once again, they also acknowledged the need for randomized phylloquinone supplementation trials in children in order to confirm these observations.

There have also been a significant number of randomized controlled trials examining phylloquinone supplementation influence on bone loss, but few have shown significant evidence of protective effects. Sokoll et al (1997) examined the response of osteocalcin in healthy adult subjects to diets formulated to contain different amounts of phylloquinones, and found that just five days of dietary supplementation of an additional 320µg of phylloquinones reduced undercarboxylated osteocalcin levels of the participants by an average of 41% as compared to when the participants were consuming a mixed diet containing about 100 µg of phylloquinones.[28] Undercarboxylated osteocalcin levels were found to rise significantly upon return to the lower-phylloquinone diet, suggesting a potential role for undercarboxylated osteocalcin as a sensitive marker for vitamin K1 status in the body. Braam et al (2003) examined the effects of three years of mineral and vitamin D supplementation and combined mineral, vitamin D, and vitamin K1 supplementation on bone density in postmenopausal women between the ages of 50 and 60. The results of the study indicated that the group of women receiving the K1 supplementation had significantly reduced bone loss of the femoral neck, but found no significant positive effects on bone density of the lumbar spine.[29] The same group then examined phylloquinone supplementation in female endurance athletes over two years as that population frequently experiences low bone mass that leads to stress fractures. However, no beneficial effects on bone loss at the femoral neck were found with supplementation.[30] A 2007 double-

blind, placebo-controlled study by Bolton-Smith et al examined the effect of two years of 200 microgram/day phylloquinone supplementation combined with vitamin D and calcium on the bone health of Scottish women over age 60; one group received a placebo, another received just 200 micrograms/day phylloquinone, a third group received vitamin D and calcium, and the final group received phylloquinone, vitamin D, and calcium.[31] BMD measurements of the ultradistal radius, mid radius, and femoral necks of the subjects were taken at baseline and every six months after for the duration of the study. Significant increases in BMD at the ultradistal radius sites and significant decreases in undercarboxylated osteocalcin were found in the group supplemented with calcium, vitamin D and phylloquinone, but no significant changes were found at the femoral neck in any group and there was significant bone mineral loss at the mid-distal radius across all groups. The results of this study were therefore inconclusive, and support a need for more randomized controlled studies examining phylloquinone supplementation on bone health.

Finally, Booth et al (2008) examined the effects of supplementation of 500 micrograms/d phylloquinone plus calcium and vitamin D in 452 men and women ages 60-80 years old in a randomized, double-blind, controlled trial.[32] Total-body BMD was measured, and measurements of the femoral neck, spine, and vitamins K and D status were measured every 6-12 months. No significant differences were found between the two groups in terms of BMD measurements at any site, while the group receiving phylloquinone supplementation presented a significant reduction in percentage of undercarboxylated osteocalcin. The authors concluded that this level of phylloquinone supplementation does not provide any significant positive benefit for bone health when consuming adequate amounts of vitamin D and calcium in the diet. Furthermore, none of the aforementioned randomized controlled trials found significant positive impacts on biomarkers of bone turnover in groups receiving phylloquinone supplementation.

More recently, research interests have shifted to examining the differences in effectiveness of vitamin K1 supplementation and menaquinone supplementation on bone and vascular health, as the contribution of menaquinones to total human vitamin K status has been found to be much higher than traditionally assumed.[33] Schurgers et al conducted a comparison between absorption and efficacy of supplementation of synthetic vitamin K1 and menaquinone-7 (MK-7), a long chain menaquinone derived from various fermented foods. A fermented soybean dish, natto, was used as the source of menaquinone-7 for this study. It was found that supplementation with MK-7 led to higher and more stable serum levels than the synthetic K1 supplement, and demonstrated higher efficacy in hepatic and extrahepatic protein carboxylation.[34] Additionally, MK-7 was found to be available longer for uptake by extrahepatic tissues than the synthetic K1; it was postulated that this was due to differences in the path of absorption between menaquinones and K1. After intestinal absorption, both are taken up in the triglyceride fractions where they are able to be cleared quickly by the liver, but higher menaquinones such as MK-7 then get redistributed via LDLs and are therefore available longer for uptake by extrahepatic tissues than K1.[34] Kannelakis et al (2012) dove deeper in to examining the effects of phylloquinone or MK-7 consumption on bone metabolism through a 12-month intervention period involving enriched dairy products containing calcium, vitamin D, and either phylloquinone or MK-7. One hundred seventy-three women were split up in to a control group that received no treatment, a group consuming yogurt and milk enriched with calcium and vitamin D, and two groups consuming yogurt and milk enriched with calcium, vitamin D, and either phylloquinone or MK-7.[35] All intervention groups also received biweekly nutrition and lifestyle counseling sessions to increase awareness of overall health issues, mainly related to osteoporosis. The authors found that all three of the intervention groups had significantly

increased intakes of calcium, vitamin D, phosphorus, and magnesium from baseline, and the group receiving phylloquinone supplementation also had significantly increased dietary phylloquinone intake. The authors also reported that both supplementation of phylloquinone and MK-7 induced significant increases in lumbar spine BMD compared to the control group, as measured through DXA, and this significance remained after controlling for serum vitamin D levels and dietary calcium intake. However, there was no significant difference between the lumbar spine BMD increases between the phylloquinone and MK-7 supplemented group.

Vitamin K Regulation of RANK Expression and Osteoclast Activity

One of the key regulators of bone modeling and metabolism is the RANK protein, or receptor activator for nuclear factor κβ, in partnership with its ligand, RANKL, and its decoy receptor osteoprogeterin (OPG). RANK is a transmembrane protein that is ubiquitously expressed in skeletal muscle, thymus, liver, small intestine, adrenal gland, pancreas, prostate, epithelial cells of the mammary gland, and osteoclasts.[36] The binding of RANKL to RANK has been well established as providing the signaling cascade that drives osteoclast development and function. In particular, RANK stimulation through RANKL binding leads to the inhibition of osteoclast apoptosis and influences the fusion of osteoclast precursors to form multinucleated cells, the differentiation of these cells into mature osteoclasts, the attachment of osteoclasts to bone, and the activation of osteoclasts to resorb bone.[37] The decoy receptor OPG is a major regulator of bone resorption, as it competes with RANKL and inhibits osteoclast activation to promote bone formation. This prevents excessive bone resorption and is a critical point at which a disruption can lead to the pathogenesis of a disease state involving osteoporosis or

osteopetrosis; decreased OPG competition leads to increased bone resorption and increased OPG competition leads to increased bone formation.[37]

Because of the importance of maintaining the delicate balance between bone resorption and formation, research has shifted to examine potential dietary influences on RANK stimulation and proper function. A recent study by Wu et al examined the inhibitory effect of vitamin K1 and K2 on RANKL-induced osteoclast differentiation and bone resorption to further examine the relationship between vitamin K and bone metabolism.[22] MK-7 is thought to possibly have a direct suppressive effect on osteoclast differentiation and activity through downregulation of NFκβ activation. Through this study, it was demonstrated that MK-4 and MK-7 significantly inhibited RANKL-mediated osteoclast differentiation of bone marrow macrophages without any evidence of cytotoxicity; both forms of K2 were shown to inhibit osteoclast formation through significant suppression of specific osteoclast differentiation markers. It was also found that the same dose of vitamin K1 did not show significant inhibition of RANKL-induced osteoclast cell formation as each form of K2 did, and K1, MK-4, and MK-7 were all found to strongly inhibit osteoclastic bone resorption in a dose-dependent manner.[22] Wu et al concluded that these results showed vitamin K2 in the forms of MK-4 and MK-7 have direct inhibitory effects on RANKL-induced osteoclast differentiation, while vitamin K1 may have somewhat of a synergistic effect on osteoclast formation, and MK-4, MK-7, and K1 all show inhibitory effects on bone resorption activity after osteoclasts are formed.

Study Objectives

This study was designed to identify specific micronutrients that make significant contributions to bone mineral density in a population of physically active pre-menarcheal females.

Methods

Study Design

A cross-sectional design will be used to examine a subset of data from a larger on-going longitudinal study on bone growth and physical activity in young females.[38] The original study was designed to compare activity and bone density data between gymnast and non-gymnasts. The data used in this analysis were collected from January 2009 to September 2012 from 52 pre-menarcheal female gymnasts and non-gymnasts between the ages of 9-11. The original longitudinal study was approved by the SUNY Upstate Medical College internal review board, while the cross sectional analysis of a subset of the data was approved by the Syracuse University internal review board.

Participant Recruitment/Demographics

The on-going longitudinal study recruited participants from gymnastics clubs, private grade schools, and athletic groups in the Syracuse, NY area. Written informed consent was obtained from both the participants and their parents prior to participation in the study. At the time of enrollment, all study subjects were between 7-12 years of age (n=122, total longitudinal study). The subjects included in the present analysis consist of 52 pre-menarcheal females representing both gymnasts and non-gymnasts. The selection criteria for this analysis was limited to participants who self-reported as Tanner Stage I (Tanner breast I and Tanner pubic I) at the time of the annual DXA scan. This criterion minimizes the potentially confounding effects of estrogen on bone density and mineral concentration. Non-Caucasian subjects were not included in this analysis as the original cohort of subjects were predominantly Caucasian and the sample size for the current study was too small to account for racial variation. Anthropometric measurements and data for body composition, calcium intake, and pubertal stage were collected

on a semi-annual basis.[38] Dietary questionnaires were completed in person during the measurement sessions.

Measurements

Dietary Analysis: Harvard's Youth/Adolescent Questionnaire (YAQ) was used to quantify nutrient intakes for the target population. A validity study was previously done in which the questionnaire was administered to 261 youths (ages 9 to 18) twice at an interval of about a year, and three 24-hour dietary recalls were collected during this time.[39] Fifty-seven percent of the subjects were between the ages of 9-13, and 96% of the subjects were Caucasian, allowing the results to be applied to our study due to similar study populations. The validity of the questionnaire was tested through comparison of the average of the two YAQs and the average of the three 24-hour dietary recalls. After examining Pearson correlation coefficients, the authors concluded that the questionnaire was a valid method whose correlations against the gold standard 24-hour recall were comparable to that of adults using similar instruments.[40]

Photocopies of the questionnaire were distributed to participants at 6-month intervals during physical measurement sessions. Participants completed the questionnaire with aid from parents when necessary. Trained research assistants transferred participants' responses to actual copies of the questionnaire. Research assistants also coded sections of the questionnaire that required participants to list any dietary supplements, cold cereals, and brands of margarine that they consumed in their households. Data were analyzed in the laboratory of Helaine Rockett, MS RD FADA (Harvard University), Channing Laboratory 3rd Floor, Brigham and Women's Hospital, 181 Longwood Ave, Boston, MA 02115.

Bone Density Measurements: All subjects underwent annual scans using a Hologic Discovery A densitometer (Waltham, MA). All scans were performed by a trained technician using the standard protocol employed in the ongoing longitudinal study.[38] Associated Hologic software was used to determine BMC (bone mineral content) and areal BMD (a derived volumetric density of calcium hydroxyapatite expressed in g/cm^3). The following sites were measured:

a. *Lumbar spine*: L1-L4 (AP)

b. *Total body: total and sub-head regions* (necessary to measure body composition, AP)

Scans were analyzed by a single investigator that was blind to subject status.

Physical Activity: Physical activity levels were self-reported by participants every 6 months during a semi-annual interview session. Participants were asked to report type of activity and hours per week of participation to determine annual mean hours per week of participation in gymnastics and other organized activities for the time period leading up to and including the scans.

Physical Maturity: Tanner breast and pubic stage were self-assessed by subjects, using standard line drawings of breast and pubic hair development, as has been previously reported to be valid for this age group.[41]

Anthropometrics: Total stature and sitting height were measured using wall-mounted rulers and a right angle. Weight was measured using an electronic scale (Detecto, Webb City, MO).

Statistical Analysis

The combined diet and activity data for the sub-sample of subjects included in the current study resulted in a large data set containing many variables with a high degree of collinearity. Before any analyses relevant to the objectives of this study could be completed, it was necessary to reduce the dimensionality of the data set. Prior analyses of some aspects of these data suggested correlations existed between several of the nutritional variables and bone density measures of interest.[38,42] When evidence exists for an underlying structure to the data, factor analysis is the appropriate statistical method to reduce the number of variables included in the analysis while minimizing the loss of relevant information. The results of the factor analysis pointed to specific dietary variables as being able to explain the majority of the variance in the data set (vitamin A, beta-carotene, carotenoids, fiber, vitamin K1, and vitamin D), we decided to focus on examining the relationships between these variables and DXA derived bone mineral density for the rest of our analyses. Although other metrics were possible (e.g., calculated BMC), BMD appeared to be the common marker used in other dietary studies of mineral metabolism.

Pearson correlation coefficients and simple linear regressions were used to determine whether changes in specific micronutrient levels led to significantly higher measures of bone mineral density. Multiple regression analysis and general linear ANOVA models were used to look for potential interactions between the intake of vitamin A, K1 and D with BMD. Physical activity, participant age and weight were used as covariates for these analyses. Finally, the relationship between the intake ratio of vitamin A: vitamin D and bone mineral density was separated into quartiles and an ANOVA (GLM) was used to determine if there were significant differences between each intake quartile in terms of bone mineral density values.

Part II: Thesis Manuscript

Introduction

Vitamin A plays an important role in bone metabolism, as osteoblasts and osteoclasts both contain nuclear receptors for retinoic acid, the active form of vitamin A in the body. Epidemiological studies examining the relationship between vitamin A intake and measures of bone metabolism have produced mixed results. Some studies propose that increased vitamin A intake is related to decreased BMD and increased risk of fracture, while others have been unable to show any statistically significant relationship between vitamin A intake on bone loss or fracture risk. Some studies even suggest that an increase in vitamin A intake may be protective against bone loss.[12,13] However, it has also been well-established that vitamin A toxicity, or hypervitaminosis A, is associated with symptoms of bone abnormalities and loss.[43]

The precise mechanism through which vitamin A impacts bone metabolism has yet to be completely elucidated. It has been proposed that vitamin A is able to function through a genomic interaction to influence bone metabolism, as supported by the presence of retinoic acid receptors in osteoblasts and osteoclasts, as well as through findings of recent *in vitro* studies and studies using animal models.[44] In recent work using bone organ culture, it was shown that vitamin A was able to stimulate bone resorption and osteoclast formation through retinoic acid-stimulated increases in mRNA production and protein expression of the osteoclastogenic cytokine, RANKL.[45] Additionally, cell culture study results have pointed to the potential for an optimality curve for vitamin A intake in relation to bone health. The optimality concept is supported by evidence that retinoic acid functions to inhibit osteoblast differentiation at nanomolar concentrations, but can also stimulate osteoblast differentiation at micromolar concentrations.[44] This suggests there is an optimal concentration of retinoic acid that might balance these opposing

cell differentiation processes. These findings, along with the mixed results of epidemiological studies, prompted our interest in focusing on the potential relationships between vitamin A intake and bone mineral density in the analysis presented here. Our target study population consists of premenarcheal females. Young women at this stage of development may be sensitive to dietary factors, such as vitamin A, that could have long lasting negative impacts on bone mineral density and increases the risk of fracture later in life. Because of this, we chose to examine the relatively unexplored areas of the relationships between bone mineral density and the intake of vitamin A, beta-carotene, carotenoids, RAE, fiber, vitamin K, and vitamin D in a population of premenarcheal female gymnasts and non-gymnasts. The fat soluble vitamins are known to function as ligands for transcription factors important in regulating bone mineralization. These transcription factors work in pairs as homo or heterodimer structures, with one of the two dimer proteins being a retinoic acid receptor (RXR). Since the RXR receptor is a common protein anchor for several transcription factor structures, it is likely that more vitamin A must be ingested than vitamins D or K to form functional heterodimer units. However, what this ratio might be is unknown at this time. The final goal of the current analysis is to investigate whether bone mineral density can be used as a biomarker to estimate adequate intake ratios of preformed vitamin A with reference to other vitamins, specifically vitamin D and K.

Data Reduction and Statistical Analysis

A factor analysis was used to reduce the dimensionality of the data set and the number of variables being examined. Two rounds of factor analysis were completed. The results of the first analysis allowed us to narrow down the number of factors to be determined for the second trial. The first factor analysis included all dietary and bone variables included in the data set (see

Appendix B). Six components had Eigenvalues above 1.0 and accounted for 90% of the variance in the data. All six factors were therefore retained for the second factor analysis. After the first factor analysis, total fat, animal fat, vegetable fats, carbohydrates, protein, body fat and lean mass, and all body composition measures except for bone mineral density and bone mineral concentration of the lumbar spine were removed from the analysis to reduce the high degree of collinearity.

Factor loadings greater than 0.50, disregarding sign, were used to define the components of the second factor analysis (appendix C). Component 1 explained the majority of the variance of the data and was strongly correlated with vitamin B2, zinc, vitamin B1, niacin, folate, retinol activity equivalents (RAE), iron, vitamin B12, vitamin D, retinol, folic acid, vitamin A, beta-carotene, and carotene. The unusual mixture of fat and water soluble vitamins suggested this component represented dietary supplementation. The correlation matrix confirmed that these nutrients, which normally would not occur together in unfortified foods, were highly correlated (appendix C). Component 2 showed high correlations with tryptophan, sodium, saturated fat, monounsaturated fat, phosphorus, potassium, calcium, and lactose. These micronutrients are most likely associated with milk and nut butters. Component 3 was strongly negatively correlated with fiber, alpha-carotene, vitamin K, and manganese. Component 4 was correlated with alpha-carotene, subtotal fat mass, and subtotal lean body mass. Component 5 explained 6% of the variance and represented measures of bone mineral density and body composition; bone mineral density anchored this component. A plot was made of the first three components to see if dietary components and body composition variables separated graphically. After examining these components, we decided to focus primarily on the relationships between bone mineral

density (component 5) and the fat soluble micronutrients in components 1 (vitamin A, vitamin D, and RAE) and 3 (fiber and vitamin K).

A series of simple linear regressions were run using data from all subjects to examine the relationships between BMD and the vitamins and minerals that stood out in the preliminary factor and principal component analyses (fiber, beta-carotene, vitamin K, vitamin A, vitamin D, and carotenoids). We chose variables for the rest of our analyses that were less likely to be highly collinear and confirmed collinearity for all multiple regressions using tolerance statistics. A tolerance value below 0.20 was considered highly collinear. BMD measures were $\log_{10}$-transformed to obtain a normal distribution. The $\log_{10}$-transformed BMD values were used for all subsequent regression analyses. A simple linear regression was run to examine the relationship between bone mineral density and average weekly hours of physical activity. Finally, multiple linear regressions were run for a different response variable, namely the change in BMD between the time the participant entered the study (time 1) and the most recent bone density measurement (time 2). The change in BMD over time was regressed against the most recent reported intake of vitamins A, D, and biologically active vitamin K. Vitamins that had positive slopes in the first regression analysis were grouped together for subsequent regressions. The age at final BMD measurement, participant weight, and physical activity were used as covariates for these analyses and all variables were entered into the models at the same time to avoid biasing the results. Biologically active vitamin K1 values were determined by subtracting the intakes of dihydrophylloquinone from the overall phylloquinone intakes reported for each subject. The interaction between vitamins A, D, and the ratio of vitamin A to D intake with bone mineral density were further explored by dividing each vitamin distribution into quartiles using a nearest rank method.

Results

The output from the principal component analysis is shown in Figure 3. Only components 1, 2, and 3 are shown as these explain the majority of the variance in the data set. Four major groupings of variables resulted (A, B, D, E) and one smaller grouping (C) that could have been associated with the two adjacent groupings.

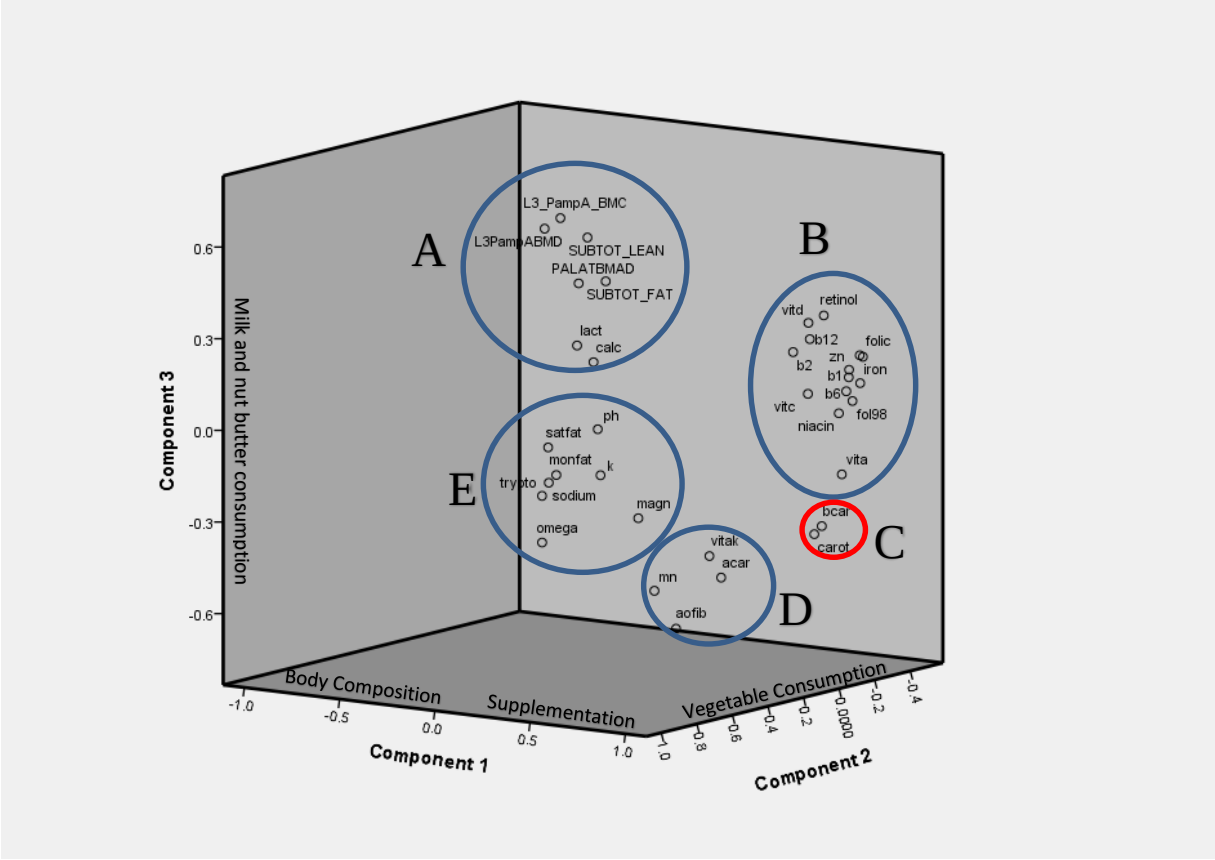


Figure 3: Graphical representation of component 3 vs component 1 vs component 2 from principal component analysis with labeled groupings. **A.** Grouping of bone measures, body composition measures, and lactose and calcium. **B.** Grouping of B vitamins, vitamins A, C, and D, and minerals, which are potentially related to supplementation. **C.** Grouping of beta-carotene and carotene, which falls between the potential supplementation grouping and a grouping of nutrients related to plant-foods. **D.** Grouping of vitamin K, alpha-carotene, manganese, and fiber, which are potentially related to plant-based intake. **E.** Grouping of nutrients potentially associated with animal products and/or nut butters, including the omega fatty acids, tryptophan, sodium, saturated fat, phosphorus, monounsaturated fat, potassium, and magnesium.

The first grouping (A) consisted of bone measures, body composition measures, lactose, and calcium; this was not surprising as lactose and calcium are generally regarded as having strong relationships to bone, as lactose enhances the absorption of calcium by enterocytes. Grouping B consisted of all the vitamins in our data set, both water soluble and fat soluble, as well as many minerals. High concentrations of both water soluble and fat soluble vitamins are unlikely to occur together in unfortified foods, leading us to conclude that this grouping was related to supplementation. Grouping C was a sub-group that consisted of just beta-carotene and carotene, and fell very close to both Grouping B and Grouping D. Grouping D consisted of vitamin K, alpha-carotene, manganese, and fiber, all of which are nutrients that may be associated with plant-based foods. Therefore, we believe that Grouping C may be associated with both supplementation as well as intake of plant-based foods. Finally, Grouping E consisted of nutrients that are commonly found in dairy products, meat, and nut butters, such as monounsaturated fat, saturated fat, sodium, tryptophan, phosphorus, magnesium, omega fatty acids, and potassium.

After carefully examining these groupings individually, we chose to focus on the fat-soluble micronutrients (vitamin A, vitamin D, carotene, and beta-carotene) as well as fiber and vitamin K, in relation to bone mineral density. Bone mineral density was chosen as the biomarker for this analyses as an extensive literature review suggested that it was the most frequently used measure to examine relationships between dietary variables and bone.

Although some studies have shown that physical activity can account for up to forty percent of bone density,[46] the correlation was not statistically significant for this data set ($r=0.157$; $\beta=0.157$; $p=0.138$, appendix D). Weak, but statistically significant inverse correlations were found to exist between dietary intakes of beta-carotene and bone mineral density ($r= -$

0.337; β= -0.337; p=0.008), carotene and bone mineral density (r= -0.344; β= -0.344; p=0.007), vitamin A and bone mineral density (r= -0.297; β= -0.297; p=0.018), and fiber (r= -0.270; β= -0.270; p=0.029). Weak, but statistically significant correlations were also found between bone mineral density and beta-carotene intake (r^2=0.113; p=0.017), bone mineral density and carotene intake (r^2=0.118; p=0.014), and bone mineral density and vitamin A intake (r^2=0.088; p=0.036). There was no statistically significant relationship between the dietary intake of vitamin K1 (phylloquinone) and bone mineral density of the lumbar spine (r= -0.083; β= -0.083; p=0.284).

The correlation analyses were not adjusted for activity level, participant age, or weight, so additional multiple regression analyses were used to further explore significant correlations. Bone mineral density increased (adjusted R^2=0.270, p=0.006, Appendix E) with the positive interaction between change in body weight (β= 0.455; p=0.022) and the intake of biologically active vitamin K (K1 minus dihydrophylloquinone β= 0.282; p=0.048, Fig. 4). Although not significant itself, vitamin D had to be present in the statistical model in order for the biologically active K1 and BMD relationship to be significant (Appendix E). The change in BMD over the period of study participation was also significantly correlated with the last reported vitamin A intake (β= 0.232; p=0.031, Fig 5) and the change in participant weight (β= 0.815; p=0.0001, Fig. 6) when adjusted for age, and physical activity (adjusted R^2=0.553, p=0.0001, Appendix E). As weight increased, the contribution of the fat soluble vitamins A and K to increased bone density became more important, while the contribution of vitamin D to the change in BMD remained the same (Fig. 7 and Fig. 8).

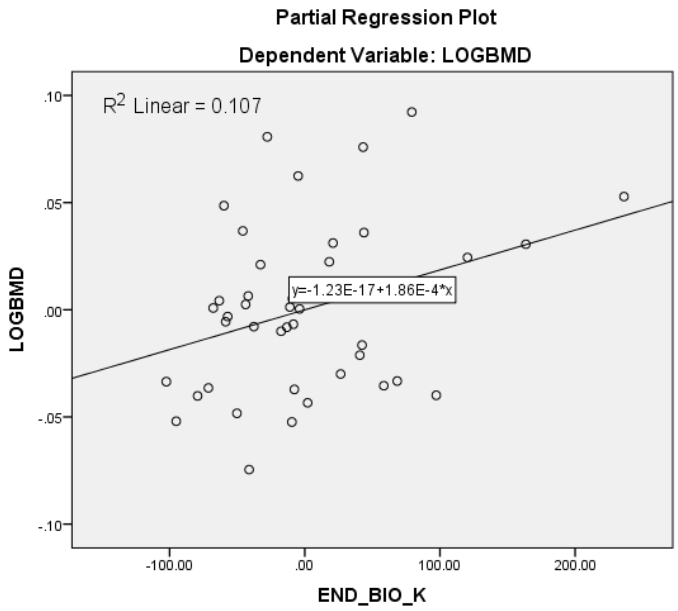


Figure 4: Bone mineral density vs intake of biologically active vitamin K. Partial regression plot from the multiple regression analysis of log normalized bone mineral density as a function of biologically active vitamin K, vitamin D, participant age, weight, and physical activity.

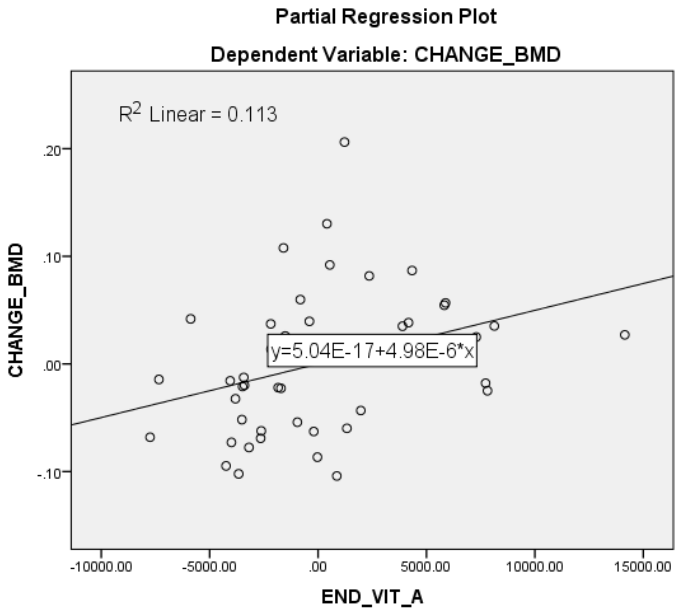


Figure 5: Change in BMD from beginning of study participation to most recent DXA scan vs most recently reported vitamin A intake, when adjusted for age and physical activity. Partial regression plot from the multiple regression analysis of the change in bone mineral density over time as a function of biologically active vitamin A, participant age, weight, and physical activity.

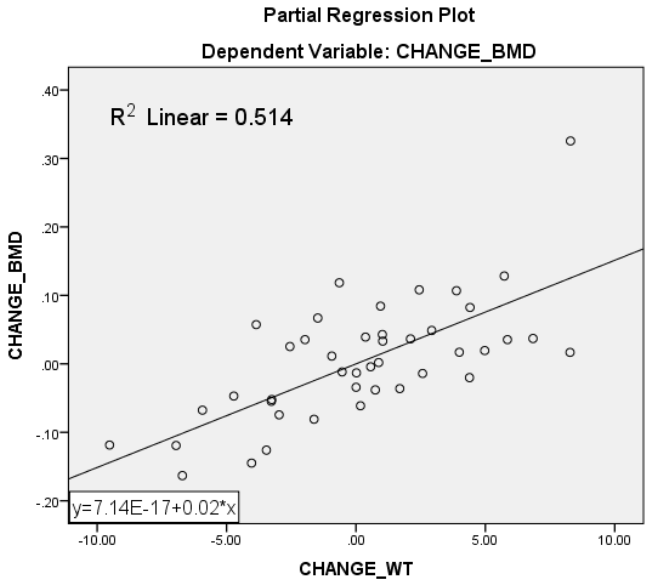


Figure 6: Partial regression plot of the change in BMD from beginning of study participation to most recent DXA scan vs change in weight from first measurement to most recent measurement, when adjusted for age and physical activity.

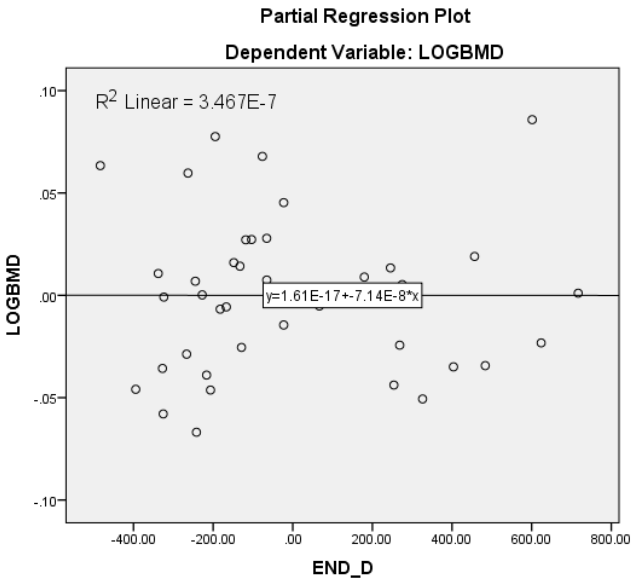


Figure 7: Partial regression plot of log normalized bone mineral density as a function of the most recently reported intake of vitamin D, with respect to change in weight.

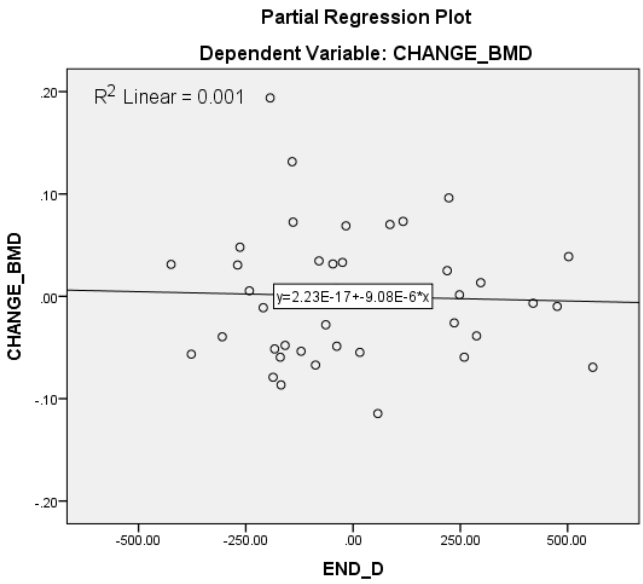


Figure 8: Partial regression plot of the change in BMD from beginning of study participation to most recent DXA scan as a function of most recently reported intake of vitamin D, participant age, weight, and physical activity.

To further explore the relationship between fat soluble vitamin intake and BMD, the individual participant intakes of vitamin A and vitamin D were assigned percentiles using a nearest rank method. The percentiles were then used to divide intake distribution into quartiles. Figure 9 depicts a boxplot of vitamin A levels in each quartile of vitamin A intake. The lowest BMD was found to coincide with the highest quartile of vitamin A intake, which contains intake values that are all above the UL for vitamin A intake for this age group (Figure 10; Appendix F). Similarly, Figure 11 depicts a boxplot of vitamin D levels separated by quartile of vitamin D intake; the lowest BMD was again associated with the highest quartile of vitamin D intake. All intakes in this quartile were above the DRI for vitamin D intake.

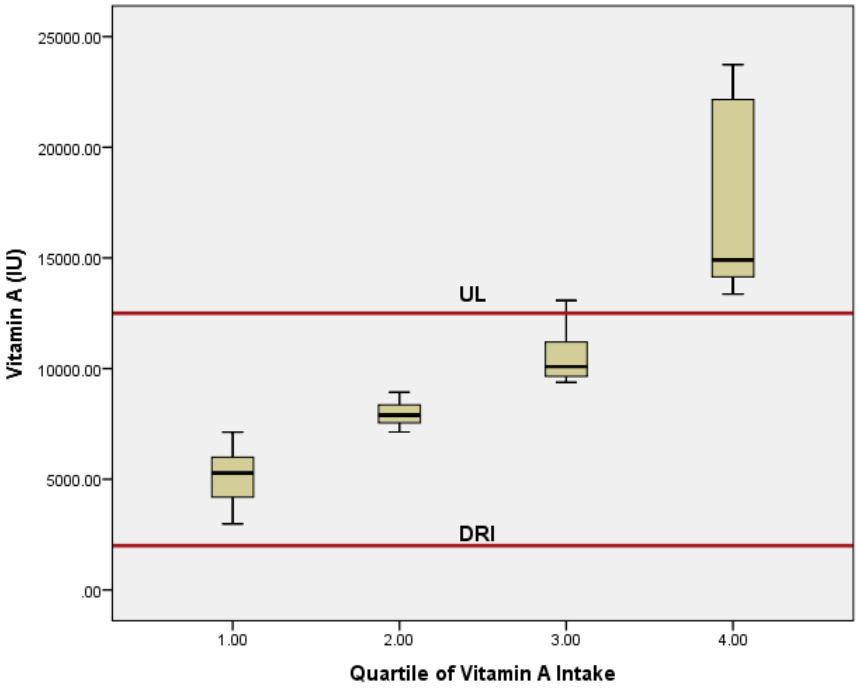


Figure 9: Vitamin A intake levels as separated by quartile of intake for all subjects. All quartiles exceeded the DRI of 2000 IU for the study population demographic. The lowest BMD were associated with the highest quartile of vitamin A intake.

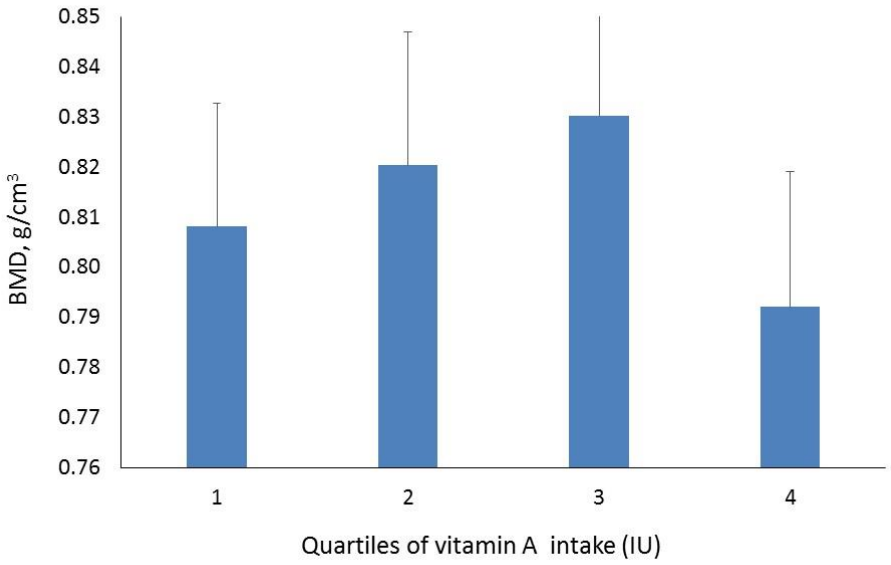


Figure 10: Bone mineral density (g cm^{-3}) as a function of vitamin A (IU) intake in a population of pre-adolescent girls. Vitamin A intake was divided into quartiles for this analysis. The reported means have been adjusted for age, body mass index, and physical activity levels; error bars are 95% confidence intervals (Appendix F). Although not statistically significant, bone mineral density was lowest at the highest level of vitamin A intake. Bone density was highest at intermediate levels of vitamin A intake.

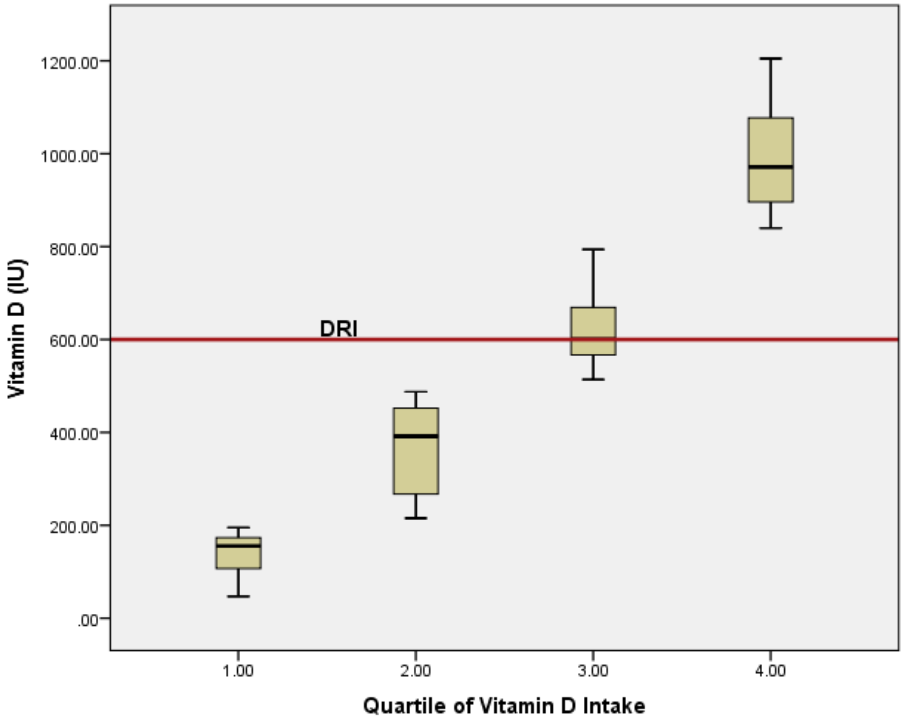


Figure 11: Vitamin D intake levels as separated by quartile of intake for all subjects. The highest quartile of vitamin D intake was associated with the lowest BMD (4st quartile of vitamin D, mean ± stdev = 0.59±0.013 g/cm^3). The highest BMD was associated with the intermediate levels of vitamin D in the 2nd and 3rd quartiles (2nd quartile, mean ± stdev = 0.64 ±0.029 g/cm^3 and 3rd quartile, mean ± stdev = 0.65 ±0.017 g/cm^3. The 3rd quartile encompassed the current daily recommended intake of vitamin D for the study population demographic.

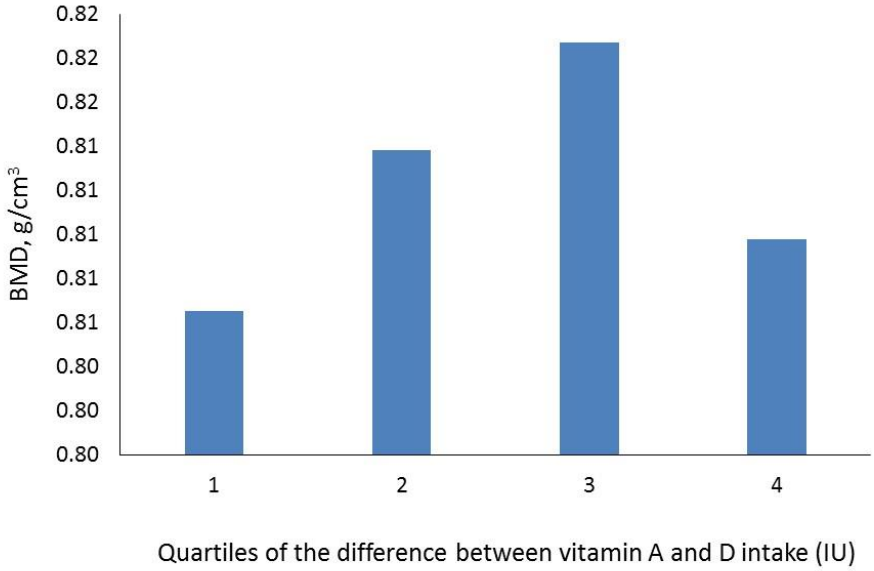


Figure 12: Bone mineral density (g cm^{-3}) as a function of the difference between vitamin A (IU) to vitamin D (IU) intake in a population of pre-adolescent girls. The difference between vitamin A and vitamin D intake was divided into quartiles for this analysis. The reported means have been adjusted for age, body mass index, and physical activity levels (Appendix F). Although not statistically significant, bone mineral density was lowest when vitamin A was either very high (4^{th} quartile, mean ± stdev = 17,317.34 ±4358.86 IU) or very low (1^{st} quartile, mean ± stdev = 5243.01 ±1230.68 IU) with respect to vitamin D intake. Intermediate levels of vitamin A:D ratio encompass the highest bone densities.

Discussion

One of the most interesting findings of our study was the statistically significant inverse association between intake of dietary fiber and bone mineral density of the lumbar spine. Fiber can affect dietary energy availability and the digestibility of complex foods as it can interact with protein and fat to decrease their digestibility. Dietary fiber also increases the bulk of intestinal contents, speeding up the transit time through the gut. This leaves less time for minerals such as calcium and magnesium to be absorbed by the body. In a study examining factors associated

with calcium absorption efficiency in pre- and perimenopausal women, Wolf et al found that fractional calcium absorption was inversely associated with dietary fiber intake.[47] Additionally, they observed that women in the lowest tertile of dietary fat to fiber ratio had a 19% lower fractional calcium absorption than women in the highest tertile. These observations may help to explain the relationship that we found between higher fiber intake and lower bone mineral density, as it implies that though calcium may be ingested in adequate amounts, it may not be efficiently absorbed if dietary fiber intake is too high.

Further evidence to support the hypothesis that high fiber intake may contribute to lower BMD comes from a recent study examining dietary intake and associations of specific nutrients with lumbar spine bone mineral density in female athletes with oligomennorhea compared to eumenorrhic athletes and nonathletes.[48] The authors observed that though oligomennorhic athletes displayed higher intakes of dietary calcium and vitamin D than the other two groups, these athletes also had higher intakes of dietary fiber, phytates, and oxalates, all of which were associated with lower BMD z scores. These effects remained even after controlling for body weight, menstrual status, calcium intake, and serum vitamin D levels. The negative effects on BMD were postulated to be associated with the effects of fiber on mineral absorption as previously described, as well as potentially through the effects of phytic acid. Intake of phytic acid was positively associated with fiber intake in this study. Phytates bind minerals, proteins, and starches, which can affect the solubility, functionality, digestion, and absorption of important bone-related minerals such as calcium and magnesium.[49] Therefore, it is possible that a simultaneous increased intake of fiber and phytic acid exerts negative impacts on bone health through the binding and subsequent decreased absorption of minerals important to bone health; this effect would potentially explain the relationship reported in the current study.

We also found a significant inverse association between vitamin A intake and bone mineral density of the lumbar spine. Retinoic acid plays a critical role in the regulation of gene expression during development, and chronic, high intakes of vitamin A are known to induce symptoms of toxicity including decreased bone mineral density. Some observational studies have found that intakes of retinol at levels slightly over the current RDA and lower than the UL were associated with poor bone mineral density and increased risk of hip fracture.[43] Promislow et al found that both high and low intakes of retinol were associated with low BMD in an elderly population, bringing up the possibility of an optimality curve for retinol intake.[50] Though this population differs greatly in age and stage of life from the participants in our study, these results are in agreement with our findings of a statistically significant association between both high and low intakes of intakesvitamin A and decreased bone mineral density of the lumbar spine.

Some of the negative consequences of excess vitamin A consumption on the body may be explained through a potential genomic effect. Excess levels of retinoic acid in the body have been postulated to increase binding of RA to retinoid receptor proteins, thus increasing the stimulation of retinoid-responsive genes.[51] This appears to be related to the postulated saturation of LRAT expression and activity in conditions of high vitamin A intake, particularly with preformed vitamin A. LRAT, or lecithin-retinol acyltransferase, is responsible for esterifying retinol in the liver that is bound to cellular retinol binding protein I (CRBP-I) in order to be able to store it. If LRAT becomes saturated, circulating levels of retinol increase; this can be problematic, as it can lead to inappropriate gene expression, the induction of apoptosis for some cells, and aberrant cell differentiation.[51] Additionally, retinoic acid stimulates the formation of osteoclasts and inhibits the activity of osteoblasts, so increased levels of retinoic acid in the bloodstream could potentially have a negative impact on bone density through these

mechanisms.[52] Therefore, it appears that the LRAT esterification step may be of critical importance in regulating vitamin A levels, and excess intake of preformed vitamin A through supplementation could be highly detrimental to the body and bone health if vitamin A levels become too great for the enzyme to handle.

Dietary intakes of preformed vitamin A, as would be found in supplementation, or other carotenoids may also affect the intestinal absorption or conversion of beta-carotene to vitamin A, as vitamin A status impacts the conversion process. If there is too much retinoic acid present in the blood, the body employs a negative feedback loop that shuts down the conversion of beta-carotene to retinoic acid. The reverse is seen in those with poor vitamin A nutritional status, as vitamin A deficiency has recently been shown to induce the expression of BCMO1, the key enzyme that converts beta-carotene to vitamin A in the intestinal cells.[53] The same study also reported BCMO1 expression was shown to be suppressed in conditions of excess vitamin A or its active metabolites, suggesting the role of a feedback loop influenced by vitamin A status. By suppressing the conversion of beta-carotene to active vitamin A, this gives the body a mechanism to prevent the aforementioned detrimental effects of excess retinoic acid in the blood stream. However, this may be another situation where preformed vitamin A ingestion can be harmful; preformed vitamin A that is ingested would be able to bypass this conversion process entirely and would therefore be much more likely to lead to excess serum retinoic acid, especially if the LRAT enzyme becomes saturated.

Statistically significant inverse associations were found between carotenoid intake and bone mineral density as well as beta-carotene intake and bone mineral density. This is contradictory to the findings of multiple epidemiological studies showing an association between increased intake of foods containing higher amounts of carotenoids with higher BMD

levels. Yang et al (2008) also found that women with osteoporosis had lower serum concentrations of lycopene and cryptoxanthin than women with higher BMD.[54] The Framingham Osteoporosis study found similar results, as they reported that lycopene intake appeared to be protective against lumbar spine BMD loss in women over a period of four years.[55] Increased intakes of total carotenoids, beta-carotene, lycopene, lutein, and zeaxanthin were also reported to be protective against trochanter bone loss in men over the same period. Specifically, participants in the highest tertile of total carotenoid intake were associated with a 46% lowered risk of hip fracture, and those with higher lycopene intake were associated with a 34% reduction in hip fracture risk and risk of non-vertebral fracture. Thus, it appears quite possible that dietary forms of vitamin A are not equal when it comes to their influence on bone health, but extensive research is still needed in this area to elucidate the mechanisms that bring about these differences in effect.

Fiber may again play a role in helping to explain our findings that beta-carotene and carotenoids were significantly associated with decreased bone mineral density. Plant sterols and other water-soluble fibers like pectin have been shown to decrease the absorption of beta-carotene, lycopene, lutein, and tocopherols.[56] Riedl et al also found that in a study of German women, water soluble fibers like pectin, guar, and alginates reduced the bioavailability of beta-carotene by about 33 to 34%.[57] These findings are of relevance to our study as they may in part explain why increased intakes of total carotenoids and beta-carotene were associated with decreased bone mineral density when epidemiological studies have generally shown positive associations between carotenoid and beta-carotene intake and bone density; if our subjects were taking in a high level of fiber, this may have negatively impacted their absorption of beta-carotene and carotenoids. This would potentially decrease or cancel out the protective effects on

bone that have been associated with these nutrients, which would lead to lower levels of bone mineral density.

Though we expected to see a significant relationship between bone mineral density and vitamin K2 intake, the YAQ data only provide direct dietary intake values for vitamin K1. We could not, therefore, conclusively report on the intake of vitamin K2 without estimates of dietary intake of specific foods known to contain vitamin K2 (cheeses, dairy products, fermented foods). Initailly, no statistically significant correlation was found between dietary intake of vitamin K1 and bone mineral density of the lumbar spine in the total sample, which is consistent with previous observational studies examining dietary intake of phylloquinone and bone mineralization markers in a similar population of young girls.[26]

Although we did not find a statistically significant direct relationship between total vitamin K1 and bone mineral density, but we did see an intriguing interaction between increased K1 intake, increased body mass, and increased BMD over the course of the course of the study when these variable were modeled together. There are a few aspects of this relationship that warrant further investigation. First, biologically active vitamin K1 was defined here as the intake of dihydrophylloquinone subtracted from the total intake of vitamin K1. We used the resulting value for additional analysis beyond simple regression, as it has been suggested that dihydrophylloquinone, a synthetic form of phylloquinone produced during vegetable oil hydrogenation, does not have the same capacity for the gamma-carboxylation of osteocalcin as phylloquinone.[58] Booth et al reported that after short-term dietary phylloquinone restriction and subsequent repletion with either phylloquinone or dihydrophylloquinone, dihydrophylloquinone was not found to have any measurable biological effects on markers of bone turnover. Phylloquinone repletion, however, was able to restore baseline values in healthy adult males.[59]

Additionally, previous research has suggested that dihydrophylloquinone can contribute to as much as 30% of vitamin K intake in children, so we wanted to ensure that we were examining only the potential interactions of biologically active phylloquinone intake with bone metabolism by excluding the values for dihydrophylloquinone.[60]

To the best of our knowledge, this is a novel finding for this age group. The statistically significant relationship between biologically active vitamin K1 and BMD is only present when vitamin D is accounted for in the model. This implies that vitamin K1 and vitamin D may participate in an interaction that impacts bone metabolism. This is a relationship that has not been extensively explored in previous research; however, a recently proposed mechanism seems to be in line with what we have found. Masterjohn proposed that excess intake of vitamin D could induce a deficiency of vitamin K through its role in the upregulation of the expression of vitamin K-dependent proteins.[61] If the level of these proteins were to exceed the pool of vitamin K available in the body for the necessary gamma-carboxylation process, it could result in a relative deficiency of vitamin K and subsequent undercarboxylation of many vitamin K-dependent proteins. One of the major vitamin K-dependent proteins that would potentially be impacted by such an event is osteocalcin, the non-collagenous bone protein in osteoblasts that has a high affinity for mineralized bone matrix and is thought to thus be involved in bone mineralization.[62] If the glutamic acid residues on this protein are not able to undergo gamma-carboxylation by vitamin K, this could potentially lead to a negative impact on bone mineralization through a decreased affinity for bone matrix, giving a possible mechanism through which a low vitamin K to vitamin D ratio could negatively impact bone metabolism. Osteocalcin's exact role in bone metabolism has not yet been elucidated, limiting the further development of this mechanism until further research has been done to determine this protein's

precise function in bone metabolism. However, a different interaction between vitamin D and vitamin K1 that could negatively impact bone density is supported by recent findings. In a mouse model, vitamin D was found to significantly reduce vitamin K uptake in a dose dependent manner by up to 58%.[63] The authors of this paper were unable to propose a mechanism for this result, but did postulate that vitamin K and vitamin D may share common uptake pathways, suggesting that vitamin D is able to outcompete vitamin K. If this is the case, this could potentially link to the previously proposed mechanism for an interaction between vitamin K and D; increased intake of vitamin D could lead to a relative deficiency of vitamin K through upregulation of vitamin K-dependent proteins, and could also be reducing the available vitamin K through outcompeting it for uptake. This relationship clearly warrants further investigation, as our results point to the ratio of intake of vitamin K1 to vitamin D as having a sizable impact on bone density when physical activity is factored in.

The role of physical activity in any potential vitamin K and vitamin D interaction also warrants further examination due to the major role exercise plays in the bone remodeling process. We used physical activity as a covariate when analyzing the relationship between BMD and vitamins K1 and D as we were interested in examining the impact of the dietary variables without interference from the mechanical influence on bone building. It would, however, be interesting to test whether or not increased physical activity could offset some of the potential negative effects of a low ratio of vitamin K1 intake to vitamin D intake, or if the dietary interaction plays too great a role in the bone metabolism process for this to occur. Once again, this is a topic for further investigation; this information could be of great importance when determining physical activity and dietary guidelines for prepubertal females.

The final relationship that we examined was that of bone mineral density to the ratio of vitamin A to vitamin D intake. We observed curvilinear patterns of BMD across quartiles of vitamin A and across quartiles of the ratio of vitamin A:D intake. These patterns suggest there may be an optimal intake of these micronutrients with respect to each other. Again, to the best of our knowledge this is a novel finding for this age group and is an area that has not been extensively explored in research thus far. We believe that this observed relationship may in part be resulting from two potential interactions between vitamin D and vitamin A, one of which is genomic. First, this relationship may stem from a role in the previously proposed mechanism of vitamin D's interaction with vitamin K1 impacting bone mineral density. In the same paper as the aforementioned mechanism, Masterjohn also proposed that vitamin A could work to protect against vitamin D's potential negative impact on vitamin K1 through its ability to downregulate matrix Gla protein.[61] This could effectively reduce the demand for carboxylation of these Gla residues on vitamin K-dependent proteins, and therefore exert a vitamin K-sparing effect to counteract the depletion that increased levels of vitamin D are proposed to induce in this hypothetical mechanism. If this is found to be true, this may also imply that symptoms of vitamin D toxicity are actually a function of the balance between vitamins A, D, and K, and not just the level of vitamin D alone. Therefore, there is the potential for an optimality curve for the ratio between vitamin A intake to vitamin D intake in relation to bone health; instead of each of these individual vitamins displaying linear relationships with bone mineral density, our data suggests that the ratio of intake of these two vitamins needs to be considered. In particular, our results appear to show that the lowest values for bone mineral density were associated with the highest intakes of vitamin D as well as the highest intakes of vitamin A, but the second highest ratio of vitamin A to vitamin D intake. In other words, the lowest bone density values were associated

with the biggest difference between vitamin A and vitamin D intake levels. In contrast, the highest values of bone mineral density in our sample were associated with a much smaller difference between vitamin A and vitamin D intakes. This leads us to believe that very large differences in the intakes of vitamin A and vitamin D may be detrimental to bone health, while smaller differences in the intake levels of the two are potentially of benefit to bone health.

Similar findings to what we have shown here have been recently reported in two observational studies. A recent analysis of the 2008-2011 Korea National Health and Nutrition Examination Survey found that total hip and femoral neck BMD in men and lumbar spine BMD in women were positively correlated with dietary vitamin A intake in participants who had serum vitamin D levels in the uppermost tertile.[64] The authors also reported that men in the lowest tertile of vitamin D intake had lower BMD in the highest A tertile and the lowest A tertile than the middle tertile of vitamin A intake; this corroborates with our results, as we saw the lowest BMD in participants with the biggest differences in vitamin A intake to vitamin D intake. Once again, these results point to the possibility of an optimality curve for vitamin A to vitamin D intake when dealing with bone health, as it appears that the largest differences in vitamin A to vitamin D intake are related to negative bone outcomes at the lowest and highest intakes of vitamin A. Mata-Granados et al also reported similar findings in a recent cross-sectional study examining the association between vitamin D deficiency or insufficiency combined with excess vitamin A intake as a risk factor for osteoporosis in postmenopausal women.[65] The authors found that BMD measurements revealed the risk of osteoporosis to be eight times higher in women with the highest retinol levels as compared to those with the lowest retinol levels. They also found that in women with deficient levels of vitamin D, the risk for osteoporosis increased substantially in those with the highest blood levels of retinol compared to those with the lowest

levels of retinol. Again, this points towards the largest differences in vitamin A to vitamin D intake increasing the potential for negative effects on bone health. The researchers similarly concluded that "an adequate bone mineral density may depend on an optimal ratio of retinol to 25(OH)D levels as a consequence of a suitable ratio of vitamin A to vitamin D intake."[65] They proposed a genomic mechanism to explain this relationship: increased tissue levels of retinoic acid allow for greater binding to nuclear retinoic X receptor (RXR) and retinoic acid receptor (RAR), effectively limiting the availability of RXRs to bind the nuclear vitamin D receptor (VDR) and activate promotors of vitamin D responsive genes. Thus, the authors proposed that increased serum retinol levels with a vitamin D deficiency or insufficiency could be detrimental for bone and mineral metabolism by decreasing vitamin D's ability to participate in its genomic activities.

We believe that our results are in line with what was found in these studies, and would like to propose a further expansion of this potential mechanism. VDR is known to be involved in the upregulation of expression of vitamin K-dependent proteins, such as osteocalcin. Specifically, vitamin D3 is known to be the principal enhancer of osteocalcin expression after the initiation of basal transcription, and is reported to be able to increase osteocalcin gene transcription by three to five-fold.[66] In mature, non-proliferating osteoblasts, vitamin D binds to the VDR to induce a conformational change and activates its translocation to the nucleus where it promotes an association with RXR. Then, the VDR/RXR complex is able to bind to the VDRE to induce transcription. Additionally, it has been reported that vitamin D is able to regulate osteocalcin gene expression at the post-translational level through stabilization of the osteocalcin mRNA.[67] If excess RA in the body is leading to increased RXR homodimers and RXR-RAR heterodimers, this would effectively decrease the number of RXR-VDR heterodimers and

decrease VDR's gene expression activity. In this case, it is quite possible that osteocalcin expression would be negatively impacted, thus leading to a decrease in its ability to participate in bone mineralization and a possibility for negative effects on bone density. This mechanism has not yet been fully explored in research, and clearly warrants further investigation. If this mechanism is able to be confirmed, this points towards a need for greater scrutiny of preformed vitamin A supplementation in multivitamins as well as the amounts found in fortified foods, particularly in developing youth; even though our participants were not ingesting levels of preformed vitamin A at a level of toxicity, the negative relationship we observed with bone density suggests the great difference in vitamin A and vitamin D intake may be associated with altered bone metabolism anyway. This is of great concern, as bone mineral density status at this developmental stage of life is known to impact fracture risk later in life, and therefore demands immediate further investigation in order to be able to prevent future widespread bone damage.

Conclusion

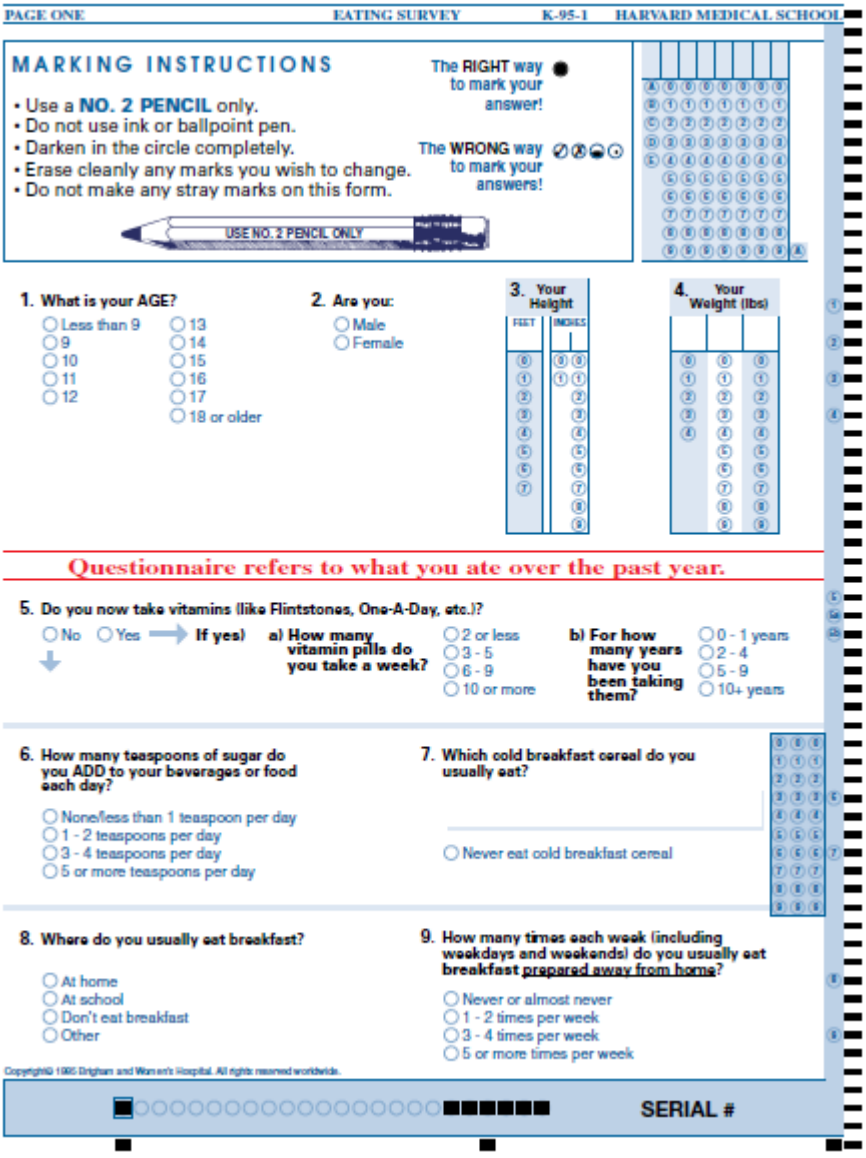
To the best of our knowledge, this is the first study to examine the relationship between the ratio of vitamin A: vitamin D intake in a premenarcheal female population. From the relationships observed here, these study results support the potential for our proposed genomic interaction between retinoic acid and vitamin D that may negatively impact markers of bone health including bone density. For premenarcheal females, this potential relationship could be of great concern, as this is a period of development in which accruing bone mineral density is highly important to future bone health. Therefore, our findings support the need for further investigation to confirm the exact mechanism behind the observed interaction that appears occur when there is a great difference in vitamin A intake and vitamin D intake, even when vitamin D intake is sufficient according to the current RDAs for this age group. Additionally, our

results also support a need for greater scrutiny of dietary supplements and foods fortified with preformed vitamin A, as in our population, ingestion of these types of substances appeared to be contributing to total vitamin A and RAE intakes well above the RDAs but below a level of toxicity.

This study also identified a statistically significant relationship between the intakes of vitamin K1, vitamin D, and BMD, which we further believe is a novel finding for this age group and gender. Once again, this is a relationship that is currently lacking a well-developed mechanism, but our results support the need for future research efforts to look at the interaction between these fat-soluble vitamins more in depth as we have shown that it is possible that increasing the ratio of intake of vitamin K1: vitamin D may positively impact bone density in this population.

Our study was not without limitations; we employed a cross-sectional design which does not allow us to determine causal relationships and we had a small sample size in a group that was homogenous in terms of socioeconomic status and geographic location. We also observed extremely high reported intakes of vitamin A that may not be consistent with average intakes for this age group across the country. These factors may limit us from being able to extend our observations to individuals of similar age and activity level outside of our sample population. Additionally, we did not have access to biomarkers for nutrient intake, and instead just examined the results of a food frequency questionnaire. Though this dietary assessment tool did allow us to get an overall general picture of dietary intakes for our subjects, it relies on accuracy of self-reporting and may affected by seasonality and recent changes in dietary pattern depending on when the questionnaire is completed.

Appendix A



PAGE ONE EATING SURVEY K-95-1 HARVARD MEDICAL SCHOOL

MARKING INSTRUCTIONS

- Use a **NO. 2 PENCIL** only.
- Do not use ink or ballpoint pen.
- Darken in the circle completely.
- Erase cleanly any marks you wish to change.
- Do not make any stray marks on this form.

The **RIGHT** way to mark your answer!

The **WRONG** way to mark your answers!

USE NO. 2 PENCIL ONLY

1. What is your AGE?

Less than 9 · 9 · 10 · 11 · 12 · 13 · 14 · 15 · 16 · 17 · 18 or older

2. Are you:

Male · Female

3. Your Height (FEET / INCHES)

4. Your Weight (lbs)

Questionnaire refers to what you ate over the past year.

5. Do you now take vitamins (like Flintstones, One-A-Day, etc.)?

No · Yes → **If yes)** **a) How many vitamin pills do you take a week?** 2 or less · 3 - 5 · 6 - 9 · 10 or more

b) For how many years have you been taking them? 0 - 1 years · 2 - 4 · 5 - 9 · 10+ years

6. How many teaspoons of sugar do you ADD to your beverages or food each day?

None/less than 1 teaspoon per day
1 - 2 teaspoons per day
3 - 4 teaspoons per day
5 or more teaspoons per day

7. Which cold breakfast cereal do you usually eat?

Never eat cold breakfast cereal

8. Where do you usually eat breakfast?

At home
At school
Don't eat breakfast
Other

9. How many times each week (including weekdays and weekends) do you usually eat breakfast prepared away from home?

Never or almost never
1 - 2 times per week
3 - 4 times per week
5 or more times per week

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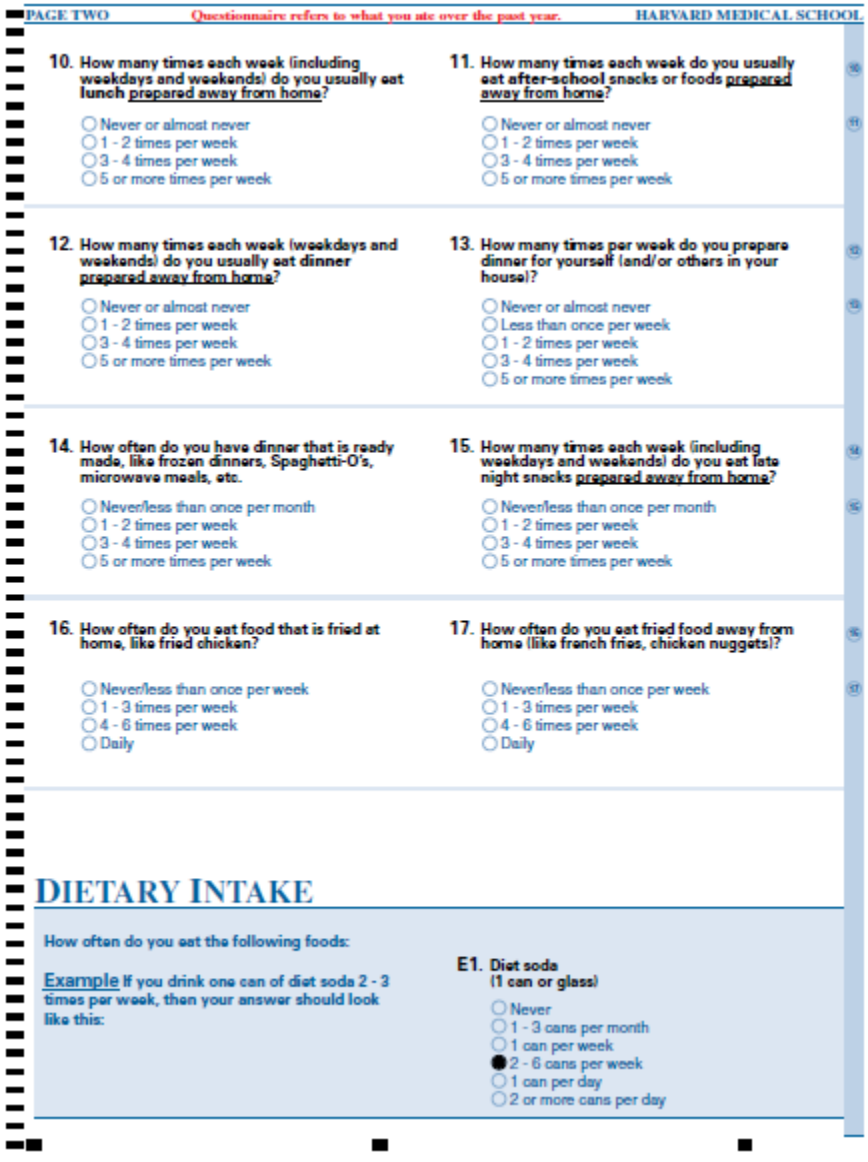


PAGE TWO — Questionnaire refers to what you ate over the past year. — HARVARD MEDICAL SCHOOL

10. How many times each week (including weekdays and weekends) do you usually eat lunch prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

11. How many times each week do you usually eat after-school snacks or foods prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

12. How many times each week (weekdays and weekends) do you usually eat dinner prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

13. How many times per week do you prepare dinner for yourself (and/or others in your house)?

- ○ Never or almost never
- ○ Less than once per week
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

14. How often do you have dinner that is ready made, like frozen dinners, Spaghetti-O's, microwave meals, etc.

- ○ Never/less than once per month
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

15. How many times each week (including weekdays and weekends) do you eat late night snacks prepared away from home?

- ○ Never/less than once per month
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

16. How often do you eat food that is fried at home, like fried chicken?

- ○ Never/less than once per week
- ○ 1 - 3 times per week
- ○ 4 - 6 times per week
- ○ Daily

17. How often do you eat fried food away from home (like french fries, chicken nuggets)?

- ○ Never/less than once per week
- ○ 1 - 3 times per week
- ○ 4 - 6 times per week
- ○ Daily

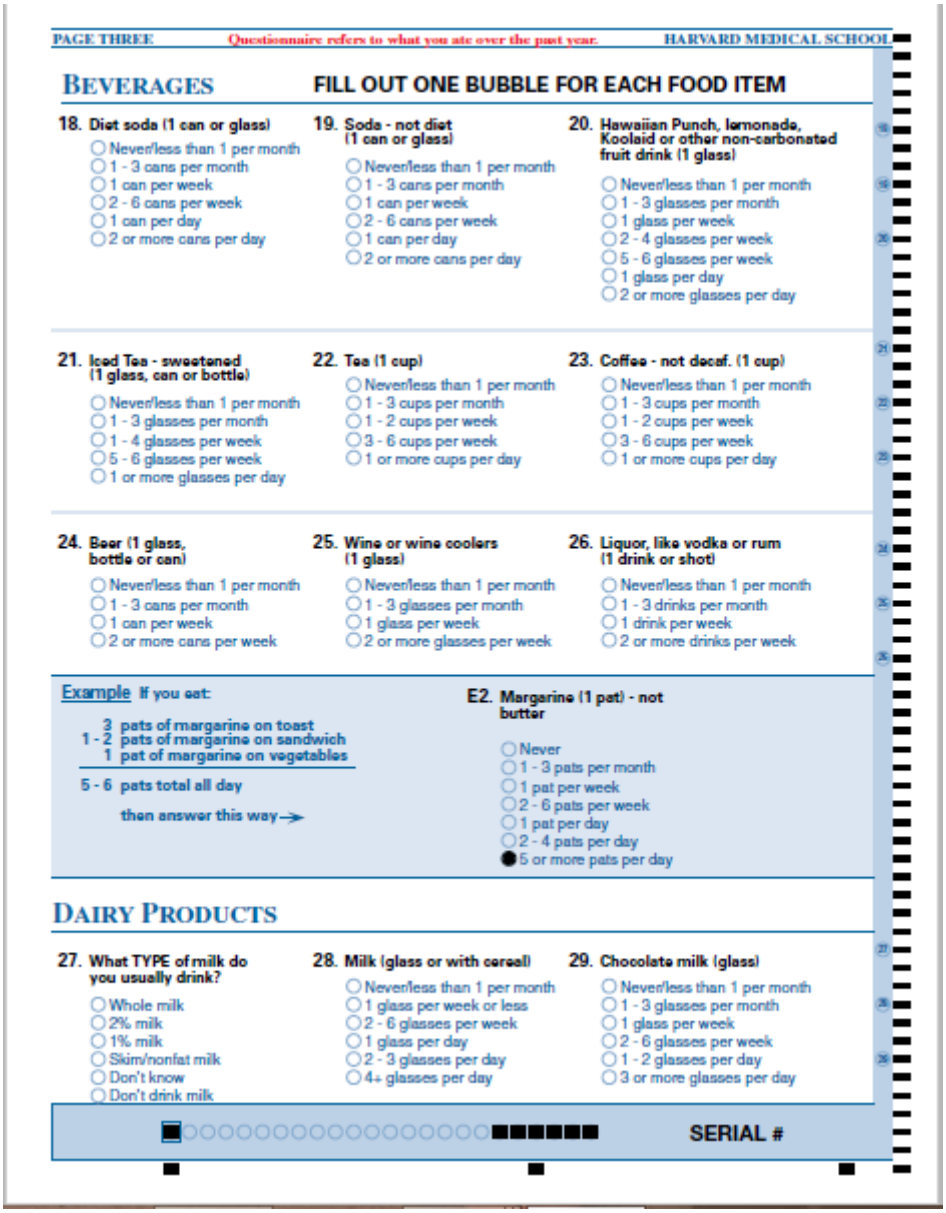
DIETARY INTAKE

How often do you eat the following foods:

Example **If you drink one can of diet soda 2 - 3 times per week, then your answer should look like this:**

E1. Diet soda (1 can or glass)

- ○ Never
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ● 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day



PAGE THREE — Questionnaire refers to what you ate over the past year. — HARVARD MEDICAL SCHOOL

BEVERAGES

FILL OUT ONE BUBBLE FOR EACH FOOD ITEM

18. Diet soda (1 can or glass)
- Never/less than 1 per month
- 1 - 3 cans per month
- 1 can per week
- 2 - 6 cans per week
- 1 can per day
- 2 or more cans per day

19. Soda - not diet (1 can or glass)
- Never/less than 1 per month
- 1 - 3 cans per month
- 1 can per week
- 2 - 6 cans per week
- 1 can per day
- 2 or more cans per day

20. Hawaiian Punch, lemonade, Koolaid or other non-carbonated fruit drink (1 glass)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 glass per week
- 2 - 4 glasses per week
- 5 - 6 glasses per week
- 1 glass per day
- 2 or more glasses per day

21. Iced Tea - sweetened (1 glass, can or bottle)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 - 4 glasses per week
- 5 - 6 glasses per week
- 1 or more glasses per day

22. Tea (1 cup)
- Never/less than 1 per month
- 1 - 3 cups per month
- 1 - 2 cups per week
- 3 - 6 cups per week
- 1 or more cups per day

23. Coffee - not decaf. (1 cup)
- Never/less than 1 per month
- 1 - 3 cups per month
- 1 - 2 cups per week
- 3 - 6 cups per week
- 1 or more cups per day

24. Beer (1 glass, bottle or can)
- Never/less than 1 per month
- 1 - 3 cans per month
- 1 can per week
- 2 or more cans per week

25. Wine or wine coolers (1 glass)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 glass per week
- 2 or more glasses per week

26. Liquor, like vodka or rum (1 drink or shot)
- Never/less than 1 per month
- 1 - 3 drinks per month
- 1 drink per week
- 2 or more drinks per week

Example If you eat:

3 pats of margarine on toast
1 - 2 pats of margarine on sandwich
1 pat of margarine on vegetables

5 - 6 pats total all day

then answer this way →

E2. Margarine (1 pat) - not butter
- Never
- 1 - 3 pats per month
- 1 pat per week
- 2 - 6 pats per week
- 1 pat per day
- 2 - 4 pats per day
- ● 5 or more pats per day

DAIRY PRODUCTS

27. What TYPE of milk do you usually drink?
- Whole milk
- 2% milk
- 1% milk
- Skim/nonfat milk
- Don't know
- Don't drink milk

28. Milk (glass or with cereal)
- Never/less than 1 per month
- 1 glass per week or less
- 2 - 6 glasses per week
- 1 glass per day
- 2 - 3 glasses per day
- 4+ glasses per day

29. Chocolate milk (glass)
- Never/less than 1 per month
- 1 - 3 glasses per month
- 1 glass per week
- 2 - 6 glasses per week
- 1 - 2 glasses per day
- 3 or more glasses per day

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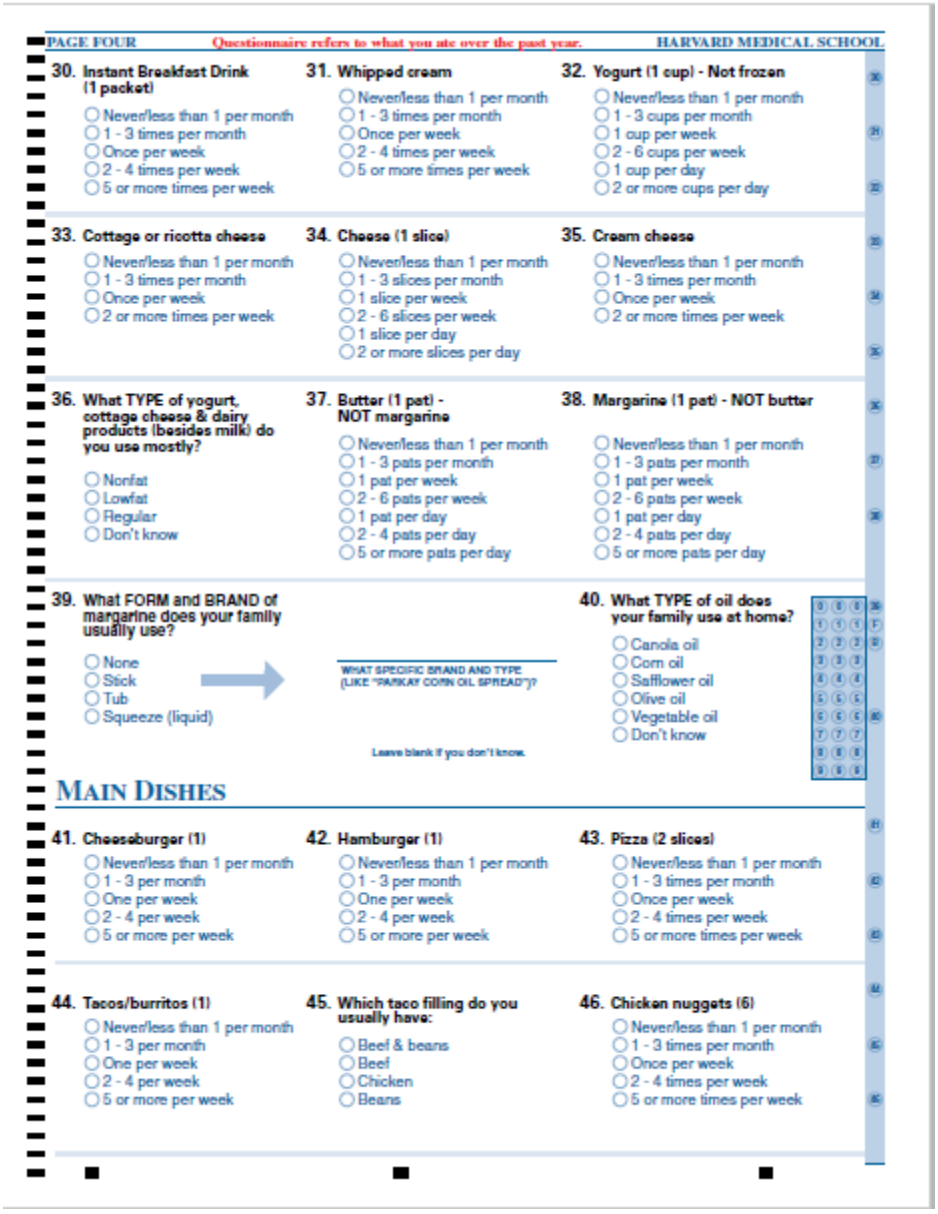


PAGE FOUR — Questionnaire refers to what you ate over the past year. — HARVARD MEDICAL SCHOOL

30. Instant Breakfast Drink (1 packet)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

31. Whipped cream
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

32. Yogurt (1 cup) - Not frozen
- Never/less than 1 per month
- 1 - 3 cups per month
- 1 cup per week
- 2 - 6 cups per week
- 1 cup per day
- 2 or more cups per day

33. Cottage or ricotta cheese
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

34. Cheese (1 slice)
- Never/less than 1 per month
- 1 - 3 slices per month
- 1 slice per week
- 2 - 6 slices per week
- 1 slice per day
- 2 or more slices per day

35. Cream cheese
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

36. What TYPE of yogurt, cottage cheese & dairy products (besides milk) do you use mostly?
- Nonfat
- Lowfat
- Regular
- Don't know

37. Butter (1 pat) - NOT margarine
- Never/less than 1 per month
- 1 - 3 pats per month
- 1 pat per week
- 2 - 6 pats per week
- 1 pat per day
- 2 - 4 pats per day
- 5 or more pats per day

38. Margarine (1 pat) - NOT butter
- Never/less than 1 per month
- 1 - 3 pats per month
- 1 pat per week
- 2 - 6 pats per week
- 1 pat per day
- 2 - 4 pats per day
- 5 or more pats per day

39. What FORM and BRAND of margarine does your family usually use?
- None
- Stick
- Tub
- Squeeze (liquid)

WHAT SPECIFIC BRAND AND TYPE (LIKE "PARKAY CORN OIL SPREAD")?

Leave blank if you don't know.

40. What TYPE of oil does your family use at home?
- Canola oil
- Corn oil
- Safflower oil
- Olive oil
- Vegetable oil
- Don't know

MAIN DISHES

41. Cheeseburger (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

42. Hamburger (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

43. Pizza (2 slices)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

44. Tacos/burritos (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

45. Which taco filling do you usually have:
- Beef & beans
- Beef
- Chicken
- Beans

46. Chicken nuggets (6)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

47. Hot dogs (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 - 4 per week
- ○ 5 or more per week

48. Peanut butter sandwich (1) (plain or with jelly, fluff, etc.)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 - 4 per week
- ○ 5 or more per week

49. Chicken or turkey sandwich (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 or more per week

50. Roast beef or ham sandwich (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 or more per week

51. Salami, bologna, or other deli meat sandwich (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 or more per week

52. Tuna sandwich (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 or more per week

53. Chicken or turkey as main dish (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

54. Fish sticks, fish cakes or fish sandwich (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

55. Fresh fish as main dish (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

56. Beef (steak, roast) or lamb as main dish (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

57. Pork or ham as main dish (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

58. Meatballs or meatloaf (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

59. Lasagna/baked ziti (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

60. Macaroni and cheese (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

61. Spaghetti with tomato sauce (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

62. Eggs (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 eggs per month
- ○ One egg per week
- ○ 2 - 4 eggs per week
- ○ 5 or more eggs per week

63. Liver: beef, calf, chicken or pork (1 serving)
- ○ Never/less than 1 per month
- ○ Less than once per month
- ○ Once per month
- ○ 2 - 3 times per month
- ○ Once per week or more

64. Shrimp, lobster, scallops (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

SERIAL #

65. French toast (2 slices)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

66. Grilled cheese (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

67. Eggrolls (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

MISCELLANEOUS FOODS

68. Brown gravy
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 - 6 times per week
- ○ Once per day
- ○ 2 or more times per day

69. Ketchup
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

70. Clear soup (with rice, noodles, vegetables) 1 bowl
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 or more bowls per week

71. Cream (milk) soups or chowder (1 bowl)
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 6 bowls per week
- ○ 1 or more bowls per day

72. Mayonnaise
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once per day

73. Low calorie/fat salad dressing
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

74. Salad dressing (not low calorie)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

75. Salsa
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

76. How much fat on your beef, pork, or lamb do you eat?
- ○ Eat all
- ○ Eat some
- ○ Eat none
- ○ Don't eat meat

77. When you have chicken or turkey, do you eat the skin?
- ○ Yes
- ○ No
- ○ Sometimes

BREADS & CEREALS

78. Cold breakfast cereal (1 bowl)
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 4 bowls per week
- ○ 5 - 7 bowls per week
- ○ 2 or more bowls per day

79. Hot breakfast cereal, like oatmeal, grits (1 bowl)
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 4 bowls per week
- ○ 5 - 7 bowls per week
- ○ 2 or more bowls per day

80. White bread, pita bread, or toast (1 slice)
- ○ Never/less than 1 per month
- ○ 1 slice per week or less
- ○ 2 - 4 slices per week
- ○ 5 - 7 slices per week
- ○ 2 - 3 slices per day
- ○ 4+ slices per day

81. Dark bread (1 slice)
- ○ Never/less than 1 per month
- ○ 1 slice per week or less
- ○ 2 - 4 slices per week
- ○ 5 - 7 slices per week
- ○ 2 - 3 slices per day
- ○ 4+ slices per day

82. English muffins or bagels (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

83. Muffin (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 muffins per month
- ○ 1 muffin per week
- ○ 2 - 4 muffins per week
- ○ 5 or more muffins per week

84. Cornbread (1 square)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more per week

85. Biscuit/roll (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

86. Rice
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

87. Noodles, pasta
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

88. Tortilla - no filling (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

89. Other grains, like kasha, couscous, bulgur
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

90. Pancakes (2) or waffles (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

91. French fries (large order)
- ○ Never/less than 1 per month
- ○ 1 - 3 orders per month
- ○ 1 order per week
- ○ 2 - 4 orders per week
- ○ 5 or more orders per week

92. Potatoes - baked, boiled, mashed
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

FRUITS & VEGETABLES

93. Raisins (small pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

94. Grapes (bunch)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

95. Bananas (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

96. Cantaloupe, melons (1/4 melon)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 per week
- ○ 2 or more times per week

97. Apples (1) or applesauce
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

98. Pears (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

99. Oranges (1), grapefruit (1/2)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

100. Strawberries
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

101. Peaches, plums, apricots (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 or more per week

102. Orange juice (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

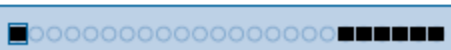
103. Apple juice and other fruit juices (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

104. Tomatoes (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

105. Tomato/spaghetti sauce
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

106. Tofu
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

107. String beans
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week



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108. Beans/lentils/soybeans
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 - 6 times per week
- ○ Once per day

109. Broccoli
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

110. Beets (not greens)
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 or more times per week

111. Corn
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

112. Peas or lima beans
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

113. Mixed vegetables
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

114. Spinach
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

115. Greens/kale
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

116. Green/red peppers
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

117. Yams/sweet potatoes (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

118. Zucchini, summer squash, eggplant
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

119. Carrots, cooked
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

120. Carrots, raw
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

121. Celery
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

122. Lettuce/tossed salad
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ One or more per day

123. Coleslaw
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

124. Potato salad
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

Think about your usual snacks. How often do you eat each type of snack food.

Example If you eat poptarts rarely (about 6 per year) then your answer should look like this:

E3. Poptarts (1)
- ● Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 - 6 per week
- ○ 1 or more per day

SNACK FOODS/DESSERTS

125. Fill in the number of snacks (food or drinks) eaten on school days and weekends/vacation days.

Snacks	School Days					Vacation/Weekend Days				
	NONE	1	2	3	4 OR MORE	NONE	1	2	3	4 OR MORE
Between breakfast and lunch	○	○	○	○	○	○	○	○	○	○
After lunch, before dinner	○	○	○	○	○	○	○	○	○	○
After dinner	○	○	○	○	○	○	○	○	○	○

126. Potato chips (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

127. Corn chips/Doritos (small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

128. Nachos with cheese (1 serving)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

129. Popcorn (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

130. Pretzels (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 small bags per week
- ○ 2 or more small bags per week

131. Peanuts, nuts (1 small bag)
- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

132. Fun fruit or fruit rollups (1 pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 packs per month
- ○ 1 - 4 packs per week
- ○ 5 or more packs per week

133. Graham crackers
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

134. Crackers, like saltines or wheat thins
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

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135. Poptarts (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 poptarts per month
- ○ 1 - 6 poptarts per week
- ○ 1 or more poptarts per day

136. Cake (1 slice)
- ○ Never/less than 1 per month
- ○ 1 - 3 slices per month
- ○ 1 slice per week
- ○ 2 or more slices per week

137. Snack cakes, Twinkies (1 package)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ Once per week
- ○ 2 - 6 per week
- ○ 1 or more per day

138. Danish, sweetrolls, pastry (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

139. Donuts (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 donuts per month
- ○ 1 donut per week
- ○ 2 - 6 donuts per week
- ○ 1 or more donuts per day

140. Cookies (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 cookies per month
- ○ 1 cookie per week
- ○ 2 - 6 cookies per week
- ○ 1 - 3 cookies per day
- ○ 4 or more cookies per day

141. Brownies (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

142. Pie (1 slice)
- ○ Never/less than 1 per month
- ○ 1 - 3 slices per month
- ○ 1 slice per week
- ○ 2 or more slices per week

143. Chocolate (1 bar or packet) like Hershey's or M & M's
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

144. Other candy bars (Milky Way, Snickers)
- ○ Never/less than 1 per month
- ○ 1 - 3 candy bars per month
- ○ 1 candy bar per week
- ○ 2 - 4 candy bars per week
- ○ 5 or more candy bars per week

145. Other candy without chocolate (Skittles) (1 pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

146. Jello
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

147. Pudding
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

148. Frozen yogurt
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

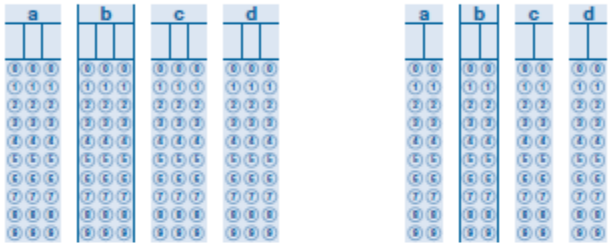
149. Ice cream
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

150. Milkshake or frappe (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 or more per week

151. Popsicles
- ○ Never/less than 1 per month
- ○ 1 - 3 popsicles per month
- ○ 1 popsicle per week
- ○ 2 - 4 popsicles per week
- ○ 5 or more popsicles per week

152. Please list any other foods that you usually eat at least once per week that are not listed (for example, coconut, hummus, falafel, chili, plantains, mangoes, etc. . .)

FOODS	HOW OFTEN?
a) _____	a) _____
b) _____	b) _____
c) _____	c) _____
d) _____	d) _____



THANK YOU FOR COMPLETING THIS SURVEY!

SERIAL #

Appendix B

Variables Included in Initial Principal Component Analysis

Dietary:

Vitamin K, Vitamin A, Retinol, Carotene, Alpha-carotene, Beta-carotene, Age, Weight, BMI, Calories, Protein, Animal Fat, Vegetable Fat, Total Fat, Carbohydrates, Fiber, Calcium, Iron, Magnesium, Phosphorus, Potassium, Zinc, Vitamin C, Vitamin D, Saturated Fat, Monounsaturated Fat, Sodium, Vitamin B1, Vitamin B2, Niacin, Vitamin B6, Folate, Vitamin B12, Lactose, Tryptophan, Manganese, Omega Fatty Acids, Folic Acid

Bone:

Postero-Anterior Bone Mineral Concentration of L3 Lumbar Spine, Postero-Anterior Bone Mineral Density of L3 Lumbar Spine, Postero-Anterior Width of L3 Lumbar Spine, Postero-Anterior Area of L3 Lumbar Spine

Appendix C

PCA Analysis

a) Correlation matrix including all variables

	aofib	vitak	vita	RAE	retinol	carot	acar	bcar	calc	iron	magn	ph	k	zn	vitc	vitd
aofib	**1.000**	.457	.480	.240	.180	.537	.527	.524	.244	.339	**.780**	.479	**.630**	.306	.288	.181
vitak	.457	**1.000**	.572	.261	.123	**.677**	.511	**.687**	-.100	.255	.246	.037	.153	.254	.162	.105
vita	.480	.572	**1.000**	**.863**	**.745**	**.953**	**.721**	**.962**	.207	**.770**	.391	.253	.293	**.794**	.552	**.717**
RAE	.240	.261	**.863**	**1.000**	**.959**	**.681**	.320	**.711**	.284	**.925**	.328	.279	.234	**.952**	**.636**	**.908**
retinol	.180	.123	**.745**	**.959**	**1.000**	.512	.171	.543	.450	**.852**	.392	.421	.340	**.904**	.551	**.927**
carot	.537	**.677**	**.953**	**.681**	.512	**1.000**	**.860**	**.998**	.067	**.604**	.326	.138	.225	**.610**	.458	.506
acar	.527	.511	**.721**	.320	.171	**.860**	**1.000**	**.826**	.092	.210	.281	.130	.242	.221	.186	.198
bcar	.524	**.687**	**.962**	**.711**	.543	**.998**	**.826**	**1.000**	.062	**.639**	.323	.136	.217	**.647**	.477	.533
calc	.244	-.100	.207	.284	.450	.067	.092	.062	**1.000**	.137	**.716**	**.928**	**.840**	.297	.029	.562
iron	.339	.255	**.770**	**.925**	**.852**	**.604**	.210	**.639**	.137	**1.000**	.358	.219	.192	**.958**	**.678**	**.815**
magn	**.780**	.246	.391	.328	.392	.326	.281	.323	**.716**	.358	**1.000**	**.896**	**.937**	.429	.247	.451
ph	.479	.037	.253	.279	.421	.138	.130	.136	**.928**	.219	**.896**	**1.000**	**.949**	.360	.063	.511
k	**.630**	.153	.293	.234	.340	.225	.242	.217	**.840**	.192	**.937**	**.949**	**1.000**	.320	.186	.430
zn	.306	.254	**.794**	**.952**	**.904**	**.610**	.221	**.647**	.297	**.958**	.429	.360	.320	**1.000**	**.678**	**.896**
vitc	.288	.162	.552	**.636**	.551	.458	.186	.477	.029	**.678**	.247	.063	.186	**.678**	**1.000**	.519
vitd	.181	.105	**.717**	**.908**	**.927**	.506	.198	.533	.562	**.815**	.451	.511	.430	**.896**	.519	**1.000**
satfat	.343	.069	.037	.045	.186	-.040	-.002	-.042	**.728**	.022	**.652**	**.772**	**.742**	.123	-.119	.263
monfat	.408	.119	.019	.039	.165	-.052	-.057	-.047	**.603**	.089	**.712**	**.758**	**.729**	.179	-.047	.202
sodium	.497	.118	.021	.010	.138	-.033	-.024	-.032	**.627**	.057	**.764**	**.790**	**.772**	.131	-.039	.152
b1	.372	.221	**.767**	**.935**	**.889**	.582	.199	**.616**	.274	**.982**	.446	.341	.307	**.974**	**.668**	**.871**
b2	.356	.149	**.712**	**.882**	**.921**	.499	.198	.526	**.639**	**.836**	**.630**	**.651**	.594	**.909**	.563	**.953**
niacin	.445	.301	**.742**	**.875**	**.821**	.580	.185	**.617**	.194	**.957**	.494	.345	.338	**.952**	**.666**	**.778**
b6	.403	.291	**.771**	**.901**	**.845**	**.607**	.224	**.641**	.231	**.957**	.470	.337	.348	**.958**	**.728**	**.822**
fol98	.428	.290	**.771**	**.892**	**.829**	**.610**	.231	**.643**	.175	**.974**	.431	.272	.275	**.937**	**.711**	**.789**
b12	.264	.170	**.703**	**.885**	**.927**	.485	.143	.518	.412	**.845**	.477	.472	.420	**.886**	.545	**.870**
lact	.136	-.131	.137	.203	.374	.014	.077	.006	**.970**	.026	**.622**	**.863**	**.790**	.197	-.033	.521
trypto	.560	.122	.041	-.013	.111	.003	.051	-.001	**.695**	.034	**.849**	**.876**	**.868**	.122	-.124	.186
mn	**.879**	.281	.264	.130	.096	.290	.260	.283	.153	.325	**.749**	.423	.542	.250	.318	.062
omega	.414	.309	-.060	-.252	-.265	.038	.101	.031	.044	-.173	.409	.262	.343	-.171	-.107	-.248
folic	.245	.213	**.734**	**.911**	**.849**	.553	.147	.591	.102	**.968**	.261	.150	.129	**.935**	**.701**	**.800**
post_BMAD	-.179	.041	-.187	-.130	-.054	-.229	-.267	-.220	.030	-.131	-.041	-.009	.008	-.139	-.042	-.097
post_ BMD	-.254	-.075	-.279	-.173	-.064	-.325	-.330	-.318	.115	-.201	-.070	.046	.028	-.216	-.100	-.132
post_BMC	-.302	-.031	-.193	-.121	-.050	-.215	-.207	-.210	.115	-.160	-.105	.039	-.016	-.154	-.148	-.067
SUBTOT_FAT	-.239	-.054	.014	.037	.077	-.011	.036	-.015	.213	-.071	-.019	.144	.153	.037	.078	.093
SUBTOT_LEAN	-.202	-.125	-.082	-.005	.056	-.116	-.068	-.117	.175	-.063	-.018	.112	.069	-.025	-.054	.032

	satfat	monfat	sodium	b1	b2	niacin	b6	fol98	b12	lact	trypto	mn	omega	folic	post_BMAD	post_BMC	post_BMD	SUBTOT_FAT	SUBTOT_LEAN
aofib	.343	.408	.497	.372	.356	.445	.403	.428	.264	.136	.560	**.879**	.414	.245	-.179	-.302	-.254	-.239	-.202
vitak	.069	.119	.118	.221	.149	.301	.291	.290	.170	-.131	.122	.281	.309	.213	.041	-.031	-.075	-.054	-.125
vita	.037	.019	.021	**.767**	**.712**	**.742**	**.771**	**.771**	**.703**	.137	.041	.264	-.060	**.734**	-.187	-.193	-.279	.014	-.082
RAE	.045	.039	.010	**.935**	**.882**	**.875**	**.901**	**.892**	**.885**	.203	-.013	.130	-.252	**.911**	-.130	-.121	-.173	.037	-.005
retinol	.186	.165	.138	**.889**	**.921**	**.821**	**.845**	**.829**	**.927**	.374	.111	.096	-.265	**.849**	-.054	-.050	-.064	.077	.056
carot	-.040	-.052	-.033	.582	.499	.580	**.607**	**.610**	.485	.014	.003	.290	.038	.553	-.229	-.215	-.325	-.011	-.116
acar	-.002	-.057	-.024	.199	.198	.185	.224	.231	.143	.077	.051	.260	.101	.147	-.267	-.207	-.330	.036	-.068
bcar	-.042	-.047	-.032	**.616**	.526	.617	**.641**	**.643**	.518	.006	-.001	.283	.031	.591	-.220	-.210	-.318	-.015	-.117
calc	**.728**	**.603**	**.627**	.274	**.639**	.194	.231	.175	.412	**.970**	**.695**	.153	.044	.102	.030	.115	.115	.213	.175
iron	.022	.089	.057	**.982**	**.836**	**.957**	**.957**	**.974**	**.845**	.026	.034	.325	-.173	**.968**	-.131	-.160	-.201	-.071	-.063
magn	**.652**	**.712**	**.764**	.446	**.630**	.494	.470	.431	.477	**.622**	**.849**	**.749**	.409	.261	-.041	-.105	-.070	-.019	-.018
ph	**.772**	**.758**	**.790**	.341	**.651**	.345	.337	.272	.472	**.863**	**.876**	.423	.262	.150	-.009	.039	.046	.144	.112
k	**.742**	**.729**	**.772**	.307	.594	.338	.348	.275	.420	**.790**	**.868**	.542	.343	.129	.008	-.016	.028	.153	.069
zn	.123	.179	.131	**.974**	**.909**	**.952**	**.958**	**.937**	**.886**	.197	.122	.250	-.171	**.935**	-.139	-.154	-.216	.037	-.025
vitc	-.119	-.047	-.039	**.668**	.563	**.666**	**.728**	**.711**	.545	-.033	-.124	.318	-.107	**.701**	-.042	-.148	-.100	.078	-.054
vitd	.263	.202	.152	**.871**	**.953**	**.778**	**.822**	**.789**	**.870**	.521	.186	.062	-.248	**.800**	-.097	-.067	-.132	.093	.032
satfat	**1.000**	**.895**	**.792**	.116	.393	.111	.104	.071	.228	**.663**	**.784**	.233	.262	-.021	-.007	-.013	.036	.098	-.022
monfat	**.895**	**1.000**	**.886**	.158	.368	.237	.172	.128	.262	.512	**.834**	.407	.352	.014	-.056	-.053	-.017	.052	-.023
sodium	**.792**	**.886**	**1.000**	.144	.345	.232	.160	.119	.239	.513	**.884**	.520	.389	-.008	-.056	.019	.074	.049	.072
b1	.116	.158	.144	**1.000**	**.903**	**.962**	**.963**	**.971**	**.876**	.159	.132	.334	-.178	**.957**	-.141	-.157	-.194	-.043	-.039
b2	.393	.368	.345	**.903**	**1.000**	**.841**	**.870**	**.841**	**.895**	.555	.367	.273	-.135	**.810**	-.069	-.064	-.095	.094	.052
niacin	.111	.237	.232	**.962**	**.841**	**1.000**	**.969**	**.958**	**.863**	.071	.218	.432	-.049	**.924**	-.161	-.201	-.225	-.044	-.078
b6	.104	.172	.160	**.963**	**.870**	**.969**	**1.000**	**.974**	**.882**	.130	.160	.367	-.091	**.955**	-.082	-.157	-.185	.011	-.045
fol98	.071	.128	.119	**.971**	**.841**	**.958**	**.974**	**1.000**	**.854**	.060	.108	.412	-.140	**.975**	-.072	-.180	-.195	-.077	-.096
b12	.228	.262	.239	**.876**	**.895**	**.863**	**.882**	**.854**	**1.000**	.345	.258	.201	-.085	**.850**	-.020	-.021	-.038	.061	.026
lact	**.663**	.512	.513	.159	.555	.071	.130	.060	.345	**1.000**	**.625**	.024	.019	.004	.072	.150	.139	.253	.187
trypto	**.784**	**.834**	**.884**	.132	.367	.218	.160	.108	.258	**.625**	**1.000**	.543	.490	-.043	-.042	.015	.038	.085	.069
mn	.233	.407	.520	.334	.273	.432	.367	.412	.201	.024	.543	**1.000**	.402	.226	-.022	-.228	-.150	-.269	-.153
omega	.262	.352	.389	-.178	-.135	-.049	-.091	-.140	-.085	.019	.490	.402	**1.000**	-.234	.089	.122	.143	-.072	.048
folic	-.021	.014	-.008	**.957**	**.810**	**.924**	**.955**	**.975**	**.850**	.004	-.043	.226	-.234	**1.000**	-.060	-.151	-.170	-.057	-.069
post_BMAD	-.007	-.056	-.056	-.141	-.069	-.161	-.082	-.072	-.020	.072	-.042	-.022	.089	-.060	**1.000**	.557	**.640**	.086	.236
post_ BMD	.036	-.017	.074	-.194	-.095	-.225	-.185	-.195	-.038	.139	.038	-.150	.143	-.170	**.640**	**.897**	**1.000**	.235	.580
post_BMC	-.013	-.053	.019	-.157	-.064	-.201	-.157	-.180	-.021	.150	.015	-.228	.122	-.151	.557	**1.000**	**.897**	.341	**.773**
SUBTOT_FAT	.098	.052	.049	-.043	.094	-.044	.011	-.077	.061	.253	.085	-.269	-.072	-.057	.086	.341	.235	**1.000**	.577
SUBTOT_LEAN	-.022	-.023	.072	-.039	.052	-.078	-.045	-.096	.026	.187	.069	-.153	.048	-.069	.236	**.773**	.580	.577	**1.000**

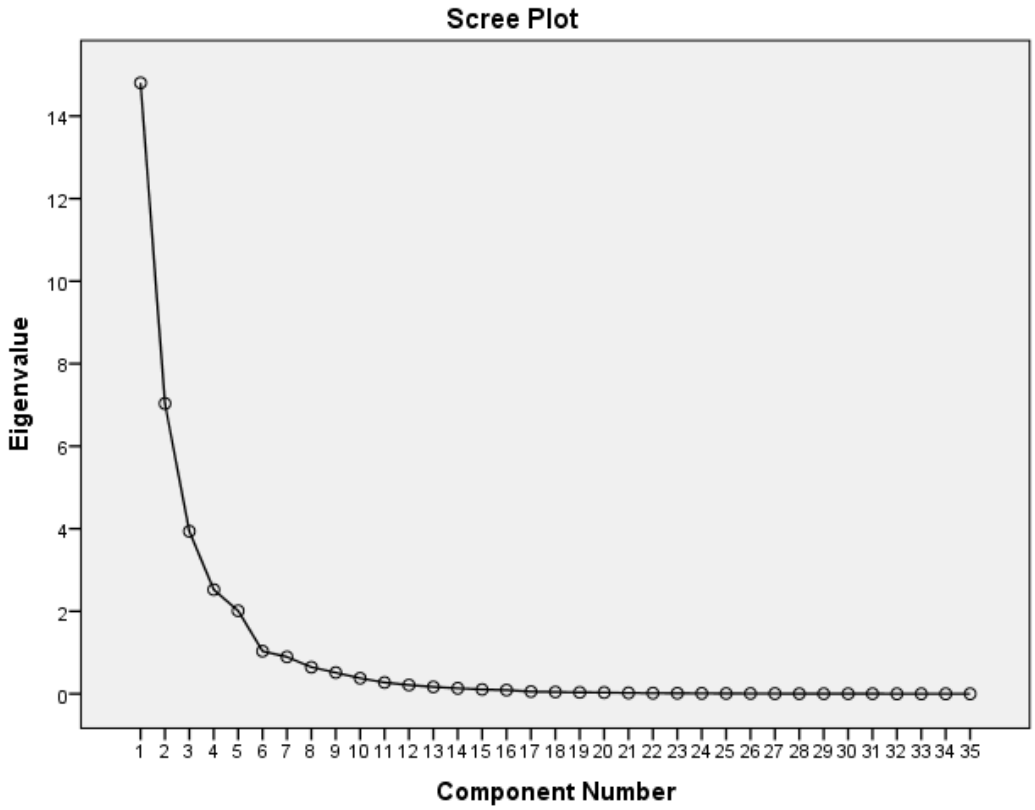
b) Total Variance Explained

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	14.805	42.301	42.301	14.805	42.301	42.301
2	7.035	20.099	62.400	7.035	20.099	62.400
3	3.937	11.249	73.649	3.937	11.249	73.649
4	2.524	7.210	80.859	2.524	7.210	80.859
5	2.012	5.747	86.607	2.012	5.747	86.607
6	1.032	2.949	89.556	1.032	2.949	89.556
7	.894	2.556	92.111			

Extraction Method: Principal Component Analysis.

c) Scree Plot



d) Component Matrix

Component Matrix[a]

	Component					
	1	2	3	4	5	6
b2	.945		.267	-.101		
zn	.934	-.245	.162			
b1	.932	-.246	.137	-.107	.136	
b6	.931	-.233			.186	
niacin	.920	-.203			.246	.118
fol98	.912	-.288			.231	
RAE	.901	-.341	.193			
iron	.892	-.353			.188	
b12	.888		.283			
vitd	.878		.335	-.127	-.208	-.110
retinol	.876	-.165	.346			
folic	.850	-.414	.173		.195	
vita	.847	-.299	-.195	.282	-.245	
bcar	.716	-.325	-.378	.397	-.259	
carot	.693	-.308	-.404	.410	-.287	
magn	.675	.662	-.213		.104	
vitc	.633	-.337			.219	.209
trypto	.367	.873	-.133			
sodium	.351	.818			.163	.121
satfat	.326	.796		-.150		
ph	.575	.783			-.130	
monfat	.357	.783		-.172	.108	
k	.575	.768				
calc	.480	.705	.289		-.345	-.135
lact	.373	.683	.333		-.433	-.182
omega		.463	-.374	.361	.345	
aofib	.552	.318	-.633	.164	.205	
post_ BMD	-.215	.236	.606	.532	.323	-.174
SUBTOT_LEAN		.166	.579	.553		.422
acar	.387	-.112	-.541	.466	-.488	
mn	.447	.327	-.516		.497	.117
post_BMC	-.184	.181	.635	.641	.177	
vitak	.352		-.469	.548		-.198
SUBTOT_FAT		.137	.437	.344	-.337	.576
post_BMAD	-.148	.116	.414	.405	.385	-.485

Extraction Method: Principal Component Analysis.

Appendix D

Simple Linear Regressions

a) BMD vs Physical Activity

Correlations

		log10BMD	ANNGymHrs
Pearson Correlation	log10BMD	1.000	.157
	ANNGymHrs	.157	1.000
Sig. (1-tailed)	log10BMD	.	.138
	ANNGymHrs	.138	.
N	log10BMD	50	50
	ANNGymHrs	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.157[a]	.025	.004	.048981717793997	.025	1.211	1	48	.277	.106

a. Predictors: (Constant), ANNGymHrs

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.003	1	.003	1.211	.277[b]
	Residual	.115	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), ANNGymHrs

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	-.215	.010		-22.563	.000			
	ANNGymHrs	.001	.001	.157	1.100	.277	.157	.157	.157

a. Dependent Variable: log10BMD

b) BMD vs Beta-carotene

Correlations

		log10BMD	bcar
Pearson Correlation	log10BMD	1.000	-.337
	bcar	-.337	1.000
Sig. (1-tailed)	log10BMD	.	.008
	bcar	.008	.
N	log10BMD	50	50
	bcar	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.337[a]	.113	.095	.046700716768054	2.015

a. Predictors: (Constant), bcar

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.013	1	.013	6.136	.017[b]
	Residual	.105	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), bcar

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.180	.013		-13.588	.000
	bcar	-7.688E-6	.000	-.337	-2.477	.017

a. Dependent Variable: log10BMD

b.

c) BMD vs Carotenoids

Correlations

		log10BMD	carot
Pearson Correlation	log10BMD	1.000	-.344
	carot	-.344	1.000
Sig. (1-tailed)	log10BMD	.	.007
	carot	.007	.
N	log10BMD	50	50
	carot	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.344[a]	.118	.100	.046568998693788	2.009

a. Predictors: (Constant), carot

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.014	1	.014	6.442	.014[b]
	Residual	.104	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), carot

Coefficients[a]

		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.180	.013		-13.819	.000
	carot	-4.262E-6	.000	-.344	-2.538	.014

a. Dependent Variable: log10BMD

d) BMD vs vitamin A

Correlations

		log10BMD	vita
Pearson Correlation	log10BMD	1.000	-.297
	vita	-.297	1.000
Sig. (1-tailed)	log10BMD	.	.018
	vita	.018	.
N	log10BMD	50	50
	vita	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.297[a]	.088	.069	.047353568360617	2.064

a. Predictors: (Constant), vita

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.010	1	.010	4.653	.036[b]
	Residual	.108	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), vita

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.179	.015		-11.913	.000
	vita	-2.831E-6	.000	-.297	-2.157	.036

a. Dependent Variable: log10BMD

e) BMD vs fiber

Correlations

		log10BMD	aofib
Pearson Correlation	log10BMD	1.000	-.270
	aofib	-.270	1.000
Sig. (1-tailed)	log10BMD	.	.029
	aofib	.029	.
N	log10BMD	50	50
	aofib	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.270[a]	.073	.054	.047754525088430	1.772

a. Predictors: (Constant), aofib

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.009	1	.009	3.773	.058[b]
	Residual	.109	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), aofib

Coefficients[a]

		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.161	.025		-6.390	.000
	aofib	-.003	.001	-.270	-1.942	.058

a. Dependent Variable: log10BMD

f) BMD vs vitamin K

Correlations

		log10BMD	vitak
Pearson Correlation	log10BMD	1.000	-.083
	vitak	-.083	1.000
Sig. (1-tailed)	log10BMD	.	.284
	vitak	.284	.
N	log10BMD	50	50
	vitak	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.083[a]	.007	-.014	.049425801297123	1.885

a. Predictors: (Constant), vitak

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.001	1	.001	.331	.568[b]
	Residual	.117	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), vitak

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.201	.015		-13.350	.000
	vitak	-6.868E-5	.000	-.083	-.575	.568

a. Dependent Variable: log10BMD

g) BMD vs Vitamin D

Correlations

		log10BMD	VitD
Pearson Correlation	log10BMD	1.000	-.143
	VitD	-.143	1.000
Sig. (1-tailed)	log10BMD	.	.162
	VitD	.162	.
N	log10BMD	50	50
	VitD	50	50

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.143[a]	.020	.000	.049089580854508	1.975

a. Predictors: (Constant), VitD

b. Dependent Variable: log10BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.002	1	.002	.995	.324[b]
	Residual	.116	48	.002		
	Total	.118	49			

a. Dependent Variable: log10BMD

b. Predictors: (Constant), VitD

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.197	.013		-15.050	.000
	VitD	-2.123E-5	.000	-.143	-.997	.324

a. Dependent Variable: log10BMD

Appendix E

Multiple Regressions

a) Change in BMD as a function of last Vitamin A measurement

b) **Variables Entered/Removed[a]**

Model	Variables Entered	Variables Removed	Method
1	gymhrs, END_AGE, END_VIT_A, CHANGE_WT[b]	.	Enter

a. Dependent Variable: CHANGE_BMD

b. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.771[a]	.594	.553	.06685

a. Predictors: (Constant), gymhrs, END_AGE, END_VIT_A, CHANGE_WT

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.255	4	.064	14.282	.000[b]
	Residual	.174	39	.004		
	Total	.430	43			

a. Dependent Variable: CHANGE_BMD

c. Predictors: (Constant), gymhrs, END_AGE, END_VIT_A, CHANGE_WT

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.017	.105		-.161	.873
	END_VIT_A	4.981E-6	.000	.232	2.232	.031
	END_AGE	-.006	.010	-.075	-.606	.548
	CHANGE_WT	.015	.002	.815	6.422	.000
	gymhrs	.001	.002	.063	.610	.545

a. Dependent Variable: CHANGE_BMD

c) Change in BMD as a function of final measurement of biologically active vitamin K (Vitamin K- dihydrophylloquinone)

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	END_BIO_K, CHANGE_WT, END_D, gymhrs, END_AGE[b]	.	Enter

a. Dependent Variable: LOGBMD

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.601[a]	.362	.270	.03996	.362	3.964	5	35	.006

a. Predictors: (Constant), END_BIO_K, CHANGE_WT, END_D, gymhrs, END_AGE

b. Dependent Variable: LOGBMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.032	5	.006	3.964	.006[b]
	Residual	.056	35	.002		
	Total	.088	40			

a. Dependent Variable: LOGBMD

b. Predictors: (Constant), END_BIO_K, CHANGE_WT, END_D, gymhrs, END_AGE

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-.306	.071		-4.325	.000	-.450	-.163		
	gymhrs	.001	.001	.084	.575	.569	-.002	.004	.862	1.160
	END_AGE	.004	.007	.100	.544	.590	-.010	.018	.545	1.836
	CHANGE_WT	.004	.002	.455	2.407	.022	.001	.007	.510	1.960
	END_D	-7.139E-8	.000	.000	-.003	.997	.000	.000	.943	1.061
	END_BIO_K	.000	.000	.282	2.045	.048	.000	.000	.962	1.040

a. Dependent Variable: LOGBMD

Tests of Between-Subjects Effects

Dependent Variable: LOGBMD

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.032[a]	5	.006	3.964	.006
Intercept	.030	1	.030	18.703	.000
END_D	1.938E-8	1	1.938E-8	.000	.997
CHANGE_WT	.009	1	.009	5.793	.022
gymhrs	.001	1	.001	.331	.569
END_AGE	.000	1	.000	.296	.590
END_BIO_K	.007	1	.007	4.183	.048
Error	.056	35	.002		
Total	1.813	41			
Corrected Total	.088	40			

a. R Squared = .362 (Adjusted R Squared = .270)

Appendix F

Quartile Analysis

a) Partial Linear Regression: Change in BMD from when subject entered study to most recent DXA scan (CHANGE_BMD) vs most recent reported average intake of vitamin A (END_VIT_A). Controlled for change in age (CHANGE_AGE) and change in weight (CHANGE_WT) from entering study until most recent data collection, age at most recent data collection date (END_AGE) and annual mean hours of physical activity (gymhrs).

Correlations

		CHANGE_BMD	gymhrs	CHANGE_AGE	CHANGE_WT	END_AGE	END_VIT_A
Pearson Correlation	CHANGE_BMD	1.000	-.057	.429	.733	.386	.129
	gymhrs	-.057	1.000	-.179	-.166	-.010	.061
	CHANGE_AGE	.429	-.179	1.000	.737	.441	-.187
	CHANGE_WT	.733	-.166	.737	1.000	.555	-.126
	END_AGE	.386	-.010	.441	.555	1.000	.040
	END_VIT_A	.129	.061	-.187	-.126	.040	1.000
Sig. (1-tailed)	CHANGE_BMD	.	.358	.002	.000	.005	.201
	gymhrs	.358	.	.122	.141	.474	.348
	CHANGE_AGE	.002	.122	.	.000	.001	.112
	CHANGE_WT	.000	.141	.000	.	.000	.207
	END_AGE	.005	.474	.001	.000	.	.397
	END_VIT_A	.201	.348	.112	.207	.397	.
N	CHANGE_BMD	44	44	44	44	44	44
	gymhrs	44	44	44	44	44	44
	CHANGE_AGE	44	44	44	44	44	44
	CHANGE_WT	44	44	44	44	44	44
	END_AGE	44	44	44	44	44	44
	END_VIT_A	44	44	44	44	44	44

Model Summary[c]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.753[a]	.567	.523	.06904	.567	12.780	4	39	.000
2	.781[b]	.610	.559	.06640	.043	4.161	1	38	.048

a. Predictors: (Constant), END_AGE, gymhrs, CHANGE_AGE, CHANGE_WT

b. Predictors: (Constant), END_AGE, gymhrs, CHANGE_AGE, CHANGE_WT, END_VIT_A

c. Dependent Variable: CHANGE_BMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.244	4	.061	12.780	.000[b]
	Residual	.186	39	.005		
	Total	.430	43			
2	Regression	.262	5	.052	11.885	.000[c]
	Residual	.168	38	.004		
	Total	.430	43			

a. Dependent Variable: CHANGE_BMD

b. Predictors: (Constant), END_AGE, gymhrs, CHANGE_AGE, CHANGE_WT

c. Predictors: (Constant), END_AGE, gymhrs, CHANGE_AGE, CHANGE_WT, END_VIT_A

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.026	.109		.235	.816	-.195	.246
	gymhrs	.001	.002	.055	.511	.613	-.003	.006
	CHANGE_AGE	-.027	.018	-.234	-1.494	.143	-.063	.009
	CHANGE_WT	.017	.003	.929	5.497	.000	.011	.024
	END_AGE	-.002	.010	-.026	-.207	.837	-.023	.018
2	(Constant)	.004	.105		.042	.967	-.209	.218
	gymhrs	.001	.002	.052	.502	.619	-.003	.005
	CHANGE_AGE	-.022	.017	-.188	-1.235	.224	-.057	.014
	CHANGE_WT	.017	.003	.942	5.788	.000	.011	.024
	END_AGE	-.005	.010	-.062	-.501	.619	-.025	.015
	END_VIT_A	4.572E-6	.000	.213	2.040	.048	.000	.000

a. Dependent Variable: CHANGE_BMD

Estimates

Dependent Variable: LOGBMD

VITAQUART	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	-.213[a]	.013	-.239	-.188
2.00	-.198[a]	.014	-.225	-.170
3.00	-.186[a]	.013	-.213	-.159
4.00	-.233[a]	.013	-.260	-.207

a. Covariates appearing in the model are evaluated at the following values:

AGE = 9.4619, BMI = 16.4236, GYMN_HOURS = 5.0362.

b) Bone mineral density reported as a function of the difference between vitamin A and vitamin D intake. Means are reported by quartile for vitamin A-vitamin D intake and are adjusted for age, body mass index, and physical activity.

Tests of Between-Subjects Effects

Dependent Variable: LOGBMD

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.020[a]	6	.003	1.353	.261
Intercept	.044	1	.044	18.321	.000
AGE	.004	1	.004	1.457	.236
BMI	.009	1	.009	3.538	.068
GYMN_HOURS	.000	1	.000	.059	.810
Quart_RATIO_A_D	.004	3	.001	.501	.684
Error	.085	35	.002		
Total	1.926	42			
Corrected Total	.104	41			

a. R Squared = .188 (Adjusted R Squared = .049)

Parameter Estimates

Dependent Variable: LOGBMD

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	-.467	.107	-4.367	.000	-.685	-.250
AGE	.008	.007	1.207	.236	-.006	.023
BMI	.011	.006	1.881	.068	-.001	.024
GYMN_HOURS	.000	.002	-.242	.810	-.004	.003
[Quart_RATIO_A_D=1.00]	-.019	.022	-.837	.408	-.064	.027
[Quart_RATIO_A_D=2.00]	.007	.023	.294	.771	-.039	.052
[Quart_RATIO_A_D=3.00]	-.001	.023	-.056	.956	-.048	.046
[Quart_RATIO_A_D=4.00]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

Estimates

Dependent Variable: LOGBMD

Quart_RATIO_A_D	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	-.223[a]	.015	-.254	-.193
2.00	-.198[a]	.016	-.230	-.166
3.00	-.206[a]	.016	-.238	-.173
4.00	-.205[a]	.016	-.237	-.172

a. Covariates appearing in the model are evaluated at the following values: AGE = 9.4954, BMI = 16.2112, GYMN_HOURS = 4.9423.

Appendix G

Daily Recommended Intakes (DRI) and Tolerable Upper Intake Levels (UL) for Vitamin A and Vitamin D for Females Ages 9-13, as Compared to Average Values for Intake in our study sample. Quartiles of intake were calculated for each vitamin separately, with the first quartile representing the lowest 25% of intake and the fourth quartile representing the highest 25% of intake.

Nutrient	**DRI: Females 9-13**	**UL**	**Average Intake: First Quartile**	**Average Intake: Second Quartile**	**Average Intake: Third Quartile**	**Average Intake: Fourth Quartile**
Vitamin A	2000 IU	5667 IU	5086 IU	7856 IU	10358 IU	17671 IU
Vitamin D	600 IU	4000 IU	138 IU	364 IU	621 IU	988 IU

References

1. Ballabriga A. Morphological and physiological changes during growth: an update. *Eur J Clin Nutr.* 2000;54 Suppl 1:S1-6.
2. Bachrach LK. Acquisition of optimal bone mass in childhood and adolescence. *Trends Endocrinol Metab.* 2001;12(1):22-28.
3. Bass SL. The prepubertal years: a uniquely opportune stage of growth when the skeleton is most responsive to exercise? *Sports Med.* 2000;30(2):73-78.
4. Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. *J Bone Miner Res.* 2014;29(2):467-478.
5. Seeman E. An Exercise in Geometry. *Journal of Bone and Mineral Research.* 2002;17(3).
6. Clarke B. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol.* 2008;3 Suppl 3:S131-139.
7. Rosenberg N, Rosenberg O, Soudry M. Osteoblasts in bone physiology-mini review. *Rambam Maimonides Med J.* 2012;3(2):e0013.
8. Olsen BR, Reginato AM, Wang W. Bone development. *Annu Rev Cell Dev Biol.* 2000;16:191-220.
9. Bellido T. Osteocyte-driven bone remodeling. *Calcif Tissue Int.* 2014;94(1):25-34.
10. Sopher AB, Fennoy I, Oberfield SE. An update on childhood bone health: mineral accrual, assessment and treatment. *Curr Opin Endocrinol Diabetes Obes.* 2015;22(1):35-40.
11. Weaver CM. Diet, gut microbiome, and bone health. *Curr Osteoporos Rep.* 2015;13(2):125-130.
12. Palacios C. The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutr.* 2006;46(8):621-628.
13. Conaway HH, Henning P, Lerner UH. Vitamin a metabolism, action, and role in skeletal homeostasis. *Endocr Rev.* 2013;34(6):766-797.
14. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J.* 2007;6:2.
15. Kajarabille N, Diaz-Castro J, Hijano S, Lopez-Frias M, Lopez-Aliaga I, Ochoa JJ. A new insight to bone turnover: role of omega-3 polyunsaturated fatty acids. *ScientificWorldJournal.* 2013;2013:589641.
16. Ross A, Taylor C, Yaktine A. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academies Press (US); 2011: http://www.ncbi.nlm.nih.gov/books/NBK56060/?report=reader#!po=0.442478.
17. Kopic S, Geibel JP. Gastric Acid, Calcium Absorption, and Their Impact on Bone Health. *Physiological Reviews.* 2013;93(1):189-268.
18. Ferland G. The discovery of vitamin K and its clinical applications. *Ann Nutr Metab.* 2012;61(3):213-218.
19. Beulens JW, Booth SL, van den Heuvel EG, Stoecklin E, Baka A, Vermeer C. The role of menaquinones (vitamin K(2)) in human health. *Br J Nutr.* 2013;110(8):1357-1368.
20. Shea MK, Booth SL. Concepts and Controversies in Evaluating Vitamin K Status in Population-Based Studies. *Nutrients.* 2016;8(1).

21. Ramotar K, Conly JM, Chubb H, Louie TJ. Production of menaquinones by intestinal anaerobes. *J Infect Dis.* 1984;150(2):213-218.
22. Wu WJ, Kim MS, Ahn BY. The inhibitory effect of vitamin K on RANKL-induced osteoclast differentiation and bone resorption. *Food Funct.* 2015;6(10):3351-3358.
23. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr.* 1999;69(1):74-79.
24. Booth SL, Tucker KL, Chen H, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr.* 2000;71(5):1201-1208.
25. Booth SL, Broe KE, Gagnon DR, et al. Vitamin K intake and bone mineral density in women and men. *Am J Clin Nutr.* 2003;77(2):512-516.
26. Kalkwarf HJ, Khoury JC, Bean J, Elliot JG. Vitamin K, bone turnover, and bone mass in girls. *Am J Clin Nutr.* 2004;80(4):1075-1080.
27. O'Connor E, Molgaard C, Michaelsen KF, Jakobsen J, Lamberg-Allardt CJ, Cashman KD. Serum percentage undercarboxylated osteocalcin, a sensitive measure of vitamin K status, and its relationship to bone health indices in Danish girls. *Br J Nutr.* 2007;97(4):661-666.
28. Sokoll LJ, Booth SL, O'Brien ME, Davidson KW, Tsaioun KI, Sadowski JA. Changes in serum osteocalcin, plasma phylloquinone, and urinary gamma-carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *Am J Clin Nutr.* 1997;65(3):779-784.
29. Braam LA, Knapen MH, Geusens P, et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. *Calcif Tissue Int.* 2003;73(1):21-26.
30. Braam LA, Knapen MH, Geusens P, Brouns F, Vermeer C. Factors affecting bone loss in female endurance athletes: a two-year follow-up study. *Am J Sports Med.* 2003;31(6):889-895.
31. Bolton-Smith C, McMurdo ME, Paterson CR, et al. Two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women. *J Bone Miner Res.* 2007;22(4):509-519.
32. Booth SL, Dallal G, Shea MK, Gundberg C, Peterson JW, Dawson-Hughes B. Effect of vitamin K supplementation on bone loss in elderly men and women. *J Clin Endocrinol Metab.* 2008;93(4):1217-1223.
33. Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis.* 2000;30(6):298-307.
34. Schurgers LJ, Teunissen KJ, Hamulyak K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood.* 2007;109(8):3279-3283.
35. Kanellakis S, Moschonis G, Tenta R, et al. Changes in parameters of bone metabolism in postmenopausal women following a 12-month intervention period using dairy products enriched with calcium, vitamin D, and phylloquinone (vitamin K(1)) or menaquinone-7 (vitamin K (2)): the Postmenopausal Health Study II. *Calcif Tissue Int.* 2012;90(4):251-262.
36. Wada T, Nakashima T, Hiroshi N, Penninger JM. RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Mol Med.* 2006;12(1):17-25.

37. Anandarajah AP. Role of RANKL in bone diseases. *Trends Endocrinol Metab.* 2009;20(2):88-94.
38. Dowthwaite JN, DiStefano JG, Ploutz-Snyder RJ, Kanaley JA, Scerpella TA. Maturity and activity-related differences in bone mineral density: Tanner I vs. II and gymnasts vs. non-gymnasts. *Bone.* 2006;39(4):895-900.
39. Rockett HR, Breitenbach M, Frazier AL, et al. Validation of a youth/adolescent food frequency questionnaire. *Prev Med.* 1997;26(6):808-816.
40. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol.* 1992;135(10):1114-1126; discussion 1127-1136.
41. Duke PM, Litt IF, Gross RT. Adolescents' Self-Assessment of Sexual Maturation. *Pediatrics.* 1980;66(6):918-920.
42. Ren J, Dowthwaite JN, Bruening KS, Scerpella TA, Brann LS. *Effects of Diet and Physical Activity on Dual Plane Dual X-Ray Absorptiometry Bone Outcomes in Pre-Pubertal Girls*: Nutrition Science, Syracuse University; 2014.
43. Ribaya-Mercado JD, Blumberg JB. Vitamin A: is it a risk factor for osteoporosis and bone fracture? *Nutr Rev.* 2007;65(10):425-438.
44. Henning P, Conaway HH, Lerner UH. Retinoid receptors in bone and their role in bone remodeling. *Front Endocrinol (Lausanne).* 2015;6:31.
45. Conaway HH, Pirhayati A, Persson E, et al. Retinoids stimulate periosteal bone resorption by enhancing the protein RANKL, a response inhibited by monomeric glucocorticoid receptor. *J Biol Chem.* 2011;286(36):31425-31436.
46. Multani NK, Kaur H, Chahal A. Impact of sporting activities on bone mineral density. *Journal of Exercise Science and Physiotherapy.* 2011;7(2):103.
47. 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.
48. Barron E, Cano Sokoloff N, Maffazioli GD, et al. Diets High in Fiber and Vegetable Protein Are Associated with Low Lumbar Bone Mineral Density in Young Athletes with Oligoamenorrhea. *J Acad Nutr Diet.* 2016;116(3):481-489.
49. Oatway L, Vasanthan T, Helm JH. Phytic Acid. *Food Reviews International.* 2001;17(4):419-431.
50. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Retinol intake and bone mineral density in the elderly: the Rancho Bernardo Study. *J Bone Miner Res.* 2002;17(8):1349-1358.
51. Ross AC, Russell RM, Miller SA, et al. Application of a key events dose-response analysis to nutrients: a case study with vitamin A (retinol). *Crit Rev Food Sci Nutr.* 2009;49(8):708-717.
52. Wu AM, Huang CQ, Lin ZK, et al. The relationship between vitamin A and risk of fracture: meta-analysis of prospective studies. *J Bone Miner Res.* 2014;29(9):2032-2039.
53. Lietz G, Lange J, Rimbach G. Molecular and dietary regulation of beta,beta-carotene 15,15'-monooxygenase 1 (BCMO1). *Arch Biochem Biophys.* 2010;502(1):8-16.
54. Yang Z, Zhang Z, Penniston KL, Binkley N, Tanumihardjo SA. Serum carotenoid concentrations in postmenopausal women from the United States with and without osteoporosis. *Int J Vitam Nutr Res.* 2008;78(3):105-111.

55. Sahni S, Mangano KM, McLean RR, Hannan MT, Kiel DP. Dietary Approaches for Bone Health: Lessons from the Framingham Osteoporosis Study. *Curr Osteoporos Rep.* 2015;13(4):245-255.
56. Granado-Lorencio F, Herrero-Barbudo C, Blanco-Navarro I, Perez-Sacristan B, Olmedilla-Alonso B. Bioavailability of carotenoids and alpha-tocopherol from fruit juices in the presence of absorption modifiers: in vitro and in vivo assessment. *Br J Nutr.* 2009;101(4):576-582.
57. Riedl J, Linseisen J, Hoffmann J, Wolfram G. Some dietary fibers reduce the absorption of carotenoids in women. *J Nutr.* 1999;129(12):2170-2176.
58. Troy LM, Jacques PF, Hannan MT, et al. Dihydrophylloquinone intake is associated with low bone mineral density in men and women. *Am J Clin Nutr.* 2007;86(2):504-508.
59. Booth SL, Lichtenstein AH, O'Brien-Morse M, et al. Effects of a hydrogenated form of vitamin K on bone formation and resorption. *Am J Clin Nutr.* 2001;74(6):783-790.
60. Booth SL, Pennington JA, Sadowski JA. Dihydro-vitamin K1: primary food sources and estimated dietary intakes in the American diet. *Lipids.* 1996;31(7):715-720.
61. Masterjohn C. Vitamin D toxicity redefined: vitamin K and the molecular mechanism. *Med Hypotheses.* 2007;68(5):1026-1034.
62. Brennan-Speranza TC, Conigrave AD. Osteocalcin: an osteoblast-derived polypeptide hormone that modulates whole body energy metabolism. *Calcif Tissue Int.* 2015;96(1):1-10.
63. Goncalves A, Roi S, Nowicki M, et al. Fat-soluble vitamin intestinal absorption: absorption sites in the intestine and interactions for absorption. *Food Chem.* 2015;172:155-160.
64. Joo NS, Yang SW, Song BC, Yeum KJ. Vitamin A intake, serum vitamin D and bone mineral density: analysis of the Korea National Health and Nutrition Examination Survey (KNHANES, 2008-2011). *Nutrients.* 2015;7(3):1716-1727.
65. Mata-Granados JM, Cuenca-Acevedo JR, Luque de Castro MD, Holick MF, Quesada-Gomez JM. Vitamin D insufficiency together with high serum levels of vitamin A increases the risk for osteoporosis in postmenopausal women. *Arch Osteoporos.* 2013;8:124.
66. Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol.* 2013;228(6):1149-1153.
67. Mosavin R, Mellon WS. Posttranscriptional regulation of osteocalcin mRNA in clonal osteoblast cells by 1,25-dihydroxyvitamin D3. *Arch Biochem Biophys.* 1996;332(1):142-152.

Vita

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Nutrition Science Education and Honors

Syracuse University
Syracuse, NY
Masters of Science, Nutrition Science
anticipated May 2016

- Awarded full graduate assistantship for 2014-2015, and 2015-2016.
- Cumulative GPA of 4.0.
- Nutrition Science Graduate Program Marshal for Falk College Convocation, May 2016
- Nutrition Science and Dietetics Graduate Research Award, 2016

University of Rochester
Rochester, NY
Bachelor of Arts in Chemistry
awarded May 18, 2014

- Cumulative GPA of 3.74, and Dean's List for 6 out of 6 Eligible Semesters
- Graduated Cum Laude, and awarded degree with High Distinction in Chemistry
- Undergraduate studies provided foundation of knowledge related to chemical and biological understandings of life processes, as well as practical skills gained through chemical and medical laboratory research.
- Science and laboratory studies and research included exploration of concepts related to nutrition at cellular levels, how psychosocial issues can impact development and personality, and biochemistry's involvement in medicine and life processes.
- *Selected courses:* Chemical Instrumentation Laboratory, Inorganic Chemistry, Physical Chemistry I (Quantum Mechanics), Organic Chemistry I with Lab, Organic Chemistry II with Honors Lab, Chemical Concepts, Systems, and Practices I & II with Labs, Bioinorganic Chemistry, Mammalian Physiology, Theories of Personality and Psychotherapy, Social and Emotional Development, Biological Chemistry, Human Anatomy, Intro to Nutrition, and Food Science.

Teaching Experience

Syracuse University
Syracuse, NY
Graduate Assistant *August 2014-Present*

- Assist in teaching graduate and upper-level undergraduate classes including nutritional biochemistry, nutrition education, community nutrition, and research methods under Dr. Margaret Voss, Dr. Jennifer Wilkins, and Dr. Beth Dixon.
- Hold weekly office hours to aide students in understanding the class material, assist with homework assignments, and review for exams.
- Responsible for grading assignments and exams, proctoring exams, and posting announcements for students on Blackboard.

University of Rochester **Rochester, NY**
Organic Chemistry I Laboratory Teaching Assistant *September-December 2012*

- Instructed students in proper laboratory techniques and safety measures.
- Taught students to write professional-grade laboratory reports; critiquing and grading work to strengthen scientific writing skills.
- Facilitated discussions of chemistry behind lab experiments to enhance student understanding of crucial organic chemistry topics.
- Evidenced understanding of proper laboratory usage and abilities to convey knowledge gained in laboratory settings to scientific audiences, as well as potential to share this information with audiences of varied levels of sophistication.

Research Experience

Syracuse University
Syracuse, NY
Thesis Research
May 2015-Present

- Guided by Dr. Margaret Voss and Dr. Lynn Brann
- Examining relationships between dietary intake, bone density, and levels of Vitamin A and vitamin K2 in young female gymnasts. In particular, looking at relationship between intake of foods containing menaquinone-4 and menaquinone-7 and bone density levels.

University of Rochester Medical Center
Rochester, NY
Research Assistant for Dr. Lei Xu *December 2012- August 2013*

- In Biomedical Genetics Department, supported Dr. Lei Xu investigations of GPR-56 protein in melanoma cells.
- Performed biochemical analysis techniques such as immunoprecipitation, western blots, and cell and tissue culture of MC-1 melanoma cell lines.
- Planned and implemented independent research entitled: "The Biochemical Characterization of GPR56," to determine ligands and potential components or associated binding partners of complex containing GPR56 in various MC-1 melanoma cell lines.
- Enhanced understanding of medical and scientific research methods and abilities to conduct literature search, transform data collected and analyzed into illustrated papers and presentations, and to share findings throughout and at completion of the project.

Internships and Volunteer and Leadership Activities

St. Vincent's Sports Performance **Indianapolis, IN**
Pre-NFL Combine Sports Nutrition Intern *January 2015*

- Worked as an intern under registered dietitian Lindsay Langford with athletes preparing for the NFL Combine. Worked in collaboration with athletic trainers, physical therapists, and strength and conditioning coaches.
- Helped personalize and create meal plans to fit each athlete's needs, assessed hydration status, enforced meal plan compliance.
- Used BodPod to assess athletes' body composition and determine approximate resting metabolic rate.
- Shadowed the dietitian in individual consults with clients, and presented to the pre-combine athletes on Sleep and Athletic Performance.
- Conducted literature searches for the dietitian on various topics including halftime nutrition and relationship of hydration status to injury.
- Created a set of nutrition tips to be distributed to USA Track and Field, and used Nutribase Software to enter and assess three-day food journals for individual clients.

Syracuse University
Syracuse, NY
Cooking on the Hillside Volunteer *September 2014-December 2014*

- Teach weekly nutrition lesson to inner-city high school students, followed by a cooking class to make a healthy meal including foods from the lesson.
- Help students understand nutrition benefits of foods while teaching them important life skills in the kitchen.

Nutrition Education and Promotion Association

- Organization promotes health and nutrition education in the Syracuse University community as well as in the city of Syracuse.

University of Rochester
Rochester, NY
Lacrosse Camp Counselor and Coach
August 2014

- Worked with 4th-8th graders of all skill levels to improve their passing and catching skills, footwork, and offensive and defensive organization, as well as overall knowledge of the game.

Member and Captain of Varsity Women's Lacrosse Team
August 2010-May 2014

- Two year captain of the team, chosen by coaching staff.
- Garnish Scholar-Athlete Award Recipient 2013 and IWLCA Academic Honor Roll 2013.

Undergraduate Co-curricular Activities

University of Rochester
Rochester, NY
Peer Career Advisor
August 2011-May 2014

- Aided undergraduate and graduate students, enhancing skills required for success in chosen career-fields.
- Assisted with resume and cover letter writing, internship or job search efforts, and use of varied online tools.

Goergen Athletic Center Fitness Center Staff and Orientation Team Member
May 2012-May 2014

- Greeted and oriented potential, new and existing members and users, ran special training and outreach, and addressed questions.

Exercise Nutrition Competencies, Curiosities and Capabilities

- Desire to explore areas of study related to sports nutrition, muscle protein synthesis, and effects of ergogenic nutritional supplements and resistance training on body composition and muscle strength.
- Capacities to conduct laboratory research to assess issues related to chemical and biochemical aspects of nutritional and exercise study.
- Experience teaching and tutoring undergraduate and graduate students in challenging scientific, nutrition-related courses.
- Career goals to become professor and researcher in the field of exercise metabolism and sport nutrition.