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Vitamin D Status and Bone Health Among Young Adult Women

Caroline Stone
University of Massachusetts Amherst

VITAMIN D STATUS AND BONE HEALTH AMONG YOUNG ADULT WOMEN

A Thesis Presented

By

CAROLINE ELIZABETH STONE

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Approved as to style and content by:

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Elizabeth Bertone-Johnson, Chair

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Marquis Hawkins, Member

_____
Susan E. Hankinson, Department Head
Biostatistics and Epidemiology

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ABSTRACT

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CAROLINE E. STONE, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Elizabeth Bertone-Johnson

Background:

Osteoporosis is estimated to affect 200 million women in the world, affecting 10% of women aged 60, 20% of women aged 70, 40% of women aged 80 and 67% of women aged 90. Osteoporosis is characterized by low bone density and increases the risk for fractured bones; however, it may be prevented with modifiable factors such as supplements, diet, and physical activity. Vitamin D deficiency leads to bone mineral density loss, as Vitamin D_3 is responsible for calcium absorption into the bones. Bone consolidation is believed to occur between 20 and 30 years old; thus, attaining peak bone mass is critical during pre-menopause.

Methods:

The relationship between vitamin D and bone mineral density has predominately been studied in postmenopausal populations. Therefore, we examined this association among 18-30 year old participants (n=271) in the cross-sectional UMass Vitamin D Status Study. The modified version of the Harvard Food Frequency Questionnaire was used to assess the average intake of vitamin D foods and supplements. Serum 25(OH)D_3 concentrations were assayed from blood samples. Bone mineral content and bone area were measured by dual-energy X-ray absorptiometry scan. Bone mineral content (BMC),

as measured in grams, provides a measure of bone mass. Bone area (BA), as measured in cm^2, reflects a two-dimensional area, which is characterized by the periphery of a bone region. We used multivariable linear regression to model the relationship between bone mineral density and bone area with sources of vitamin D after adjusting for dietary and lifestyle factors.

Results:

In the present study, the mean and standard deviation of vitamin D is 372.7 IU and 285.8 IU, respectively. For vitamin D from supplements, the mean is 140.9 IU with a standard deviation of 232.3 IU. Finally, for vitamin D from food, the mean is 231.8 IU with a standard deviation of 182.0 IU. Compared to reference values of 600 IU, these data are below the recommended daily allowance.

We did not observe an association between total vitamin D or vitamin D from foods sources with either BMC or BA. We also did not observe an association between serum 25-hydroxyvitamin D levels and BMC or BA.

Conclusion:

Future studies with larger sample sizes are warranted to validate this association among young premenopausal women.

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CHAPTER 1

INTRODUCTION

Osteoporosis is estimated to affect 200 million women in the world, affecting 10% of women aged 60, 20% of women aged 70, 40% of women aged 80 and 67% of women aged 90.[1] In 2010, there were approximately 9 million cases of osteoporosis among American women aged 50 or older. By 2020, this figure is estimated to increase to 10.5 million cases.[2] In 2005, 71% of osteoporosis-related fractures occurred among women. Of the total costs of incident fractures, over three-quarters, or $12.8 billion, were among women.[3] Risk factors for osteoporosis are family history of osteoporosis, age, low intake of calcium and vitamin D, physical inactivity, smoking, race, and female sex.[4] Menopause leads to a decrease in estrogen, a hormone that aids in the building of new bone.[5] Thus, postmenopausal women are at an increased risk of osteoporosis.

Osteoporosis is defined as bone mineral density that is 2.5 standard deviations below the young adult mean.[6] Since bone mineral density decreases gradually with age, having a high peak bone mass in young adulthood may prevent or delay osteoporosis later in life. Peak bone mass is typically achieved by the age of thirty. Prior to this age, modifiable factors such as supplements,[7] diet,[8] and physical activity/strength training[8] may increase bone mineral density. For example, a study by Alghadir et al. assessed the role of physical activity, calcium consumption and lifestyle factors and bone mineral density among 350 young adult participants. This study showed that low intake of calcium and milk, in addition to higher caffeine and carbonated beverage consumption was negatively associated with bone mineral density status.[8] Participants were classified into groups according to their bone mineral density status. In younger subjects, more than

72.6% had normal bone mineral density, while 22.6 and 4.8% had osteopenia and osteoporosis, respectively. However, among women aged 25-30, participants showed higher proportions of osteopenia (34.9%) and osteoporosis (6.9%). Compared with the younger groups of participants, 48.2% of older men and women had normal bone mineral density values, while 34.7% and 17.1% had osteopenia and osteoporosis, respectively. However, among women aged 31-45, showed the highest prevalence of osteopenia (38.5%) and osteoporosis (18.3%).[8]

In a study by Riggs et al, patterns of bone loss were studied among 187 normal participants, aged 20-89 years, and in 85 participants with vertebral fractures due to osteoporosis. Overall, the predicted mean for bone mineral density at age 90 was 47% less than the predicted mean at age 20.[9]

As mentioned previously, while vitamin D is an established risk factor for osteoporosis, it is unclear how it is associated in the attainment of peak bone mineral density in young adults. For this reason, it is necessary to examine the modifiable factors for peak bone density, specifically vitamin D.

Vitamin D has two forms; 1) Vitamin D_2 (ergocalciferol), 2) Vitamin D_3 (cholecalciferol), and several metabolites.[10] Serum 25-hydroxyvitamin D concentrations serve as a biomarker for vitamin D.[11] Vitamin D_3 is produced in two ways: ultraviolet B radiation from the sun and diet. Foods such as fatty fish (3 ounces of salmon is equivalent to 447 IU of vitamin D), milk (one cup is equivalent to 115-124 IU of vitamin D), egg yolk (1 large egg is equivalent to 41 IU of vitamin D), and beef liver (3 ounces is equal to 42 IU of vitamin D) are sources of vitamin D_3.[12] It has been suggested by researchers that approximately 5-30 minutes of sun exposure without sunscreen between 10 AM and 3

PM at least two times a week usually leads to sufficient vitamin D synthesis.[13] Vitamin D_2 is derived solely from plant sources.[14] Vitamin D_3 assists in bone mineralization and activates osteoblasts and osteoclasts. Calcitrol, the hormone derived from vitamin D, stimulates the intestines to absorb enough calcium into bone.[15] Thus, a vitamin D deficiency may lead to loss of bone mineral density.[16] Only 10% to 15% of dietary calcium and approximately 60% of phosphorus are absorbed into bones as a result of vitamin D deficiency.[16] Bone loss occurs with excessive osteoid accumulation, as well as osteomalacia, or softening of the bones.[17] Lack of sunlight exposure (i.e. through use of sunscreen or veil) and inadequate dietary vitamin D, as well as malabsorption caused by various gastrointestinal (GI) disorders may result in bone mineral loss.[17]

Physiology of the relationship between vitamin D and bone mineral density

The physiological mechanism by which vitamin D impacts bone mineral density is stronger in premenopausal women than in postmenopausal women. This is because peak rates of calcium accrual occur before the age of 30 years old.[18] Previous studies have shown that calcium accrual increases during puberty[19] and that the highest calcium accrual rates occur at a mean age of 12.5 and 14 years old in girls and boys, respectively.[20] After this rapid calcium accrual period, bone consolidation is believed to occur between the ages of 20 and 30 years old,[18] further indicating the need for our study.

Epidemiology of the relationship between vitamin D and bone mineral density

Only two prior studies investigated the association between vitamin D and bone density among premenopausal women.[21, 22] One case-control study[21] (n=100) and one

cross-sectional study[22] (n=608) examined the association between serum 25-hydroxyvitamin D and bone mineral density among 18-30 year old women. One of these studies found that vitamin D was positively associated with bone mineral density[22] while the other resulted in a null association between vitamin D and bone mineral density.[21] One of these studies adjusted for age and body mass index.[22] However, neither of these studies controlled for important confounding factors such as calcium intake and physical activity.[21, 22] Calcium and physical activity are positively associated with vitamin D and bone mineral density; therefore, not controlling for these factors would lead to an overestimate of the association between vitamin D and bone mineral density.[31, 32]

In the only cross-sectional study to assess 25-hydroxyvitamin D levels and bone mineral density in healthy premenopausal women, Adami et al. collected serum samples of women with regular monthly cyclic menses.[22] Evaluation of bone mineral density was performed via dual-energy X-ray absorptiometry (DXA) scan at the lumbar spine, the femoral neck, and total hip BMD. After adjusting for age and BMI, the regression coefficients between 25-hydroxyvitamin D concentrations and spine, femoral neck and total hip bone mineral density were +0.084 (p=0.043), +0.013 (non-significant p-value >0.1), and +0.021 (non-significant p-value >0.1) respectively.

In the only case-control study to assess 25-hydroxyvitamin D levels and bone mineral density in patients with fibromyalgia syndrome, Olama et al. collected venous blood samples.[21] Bone mineral density was measured at the lumbar spine, femoral neck, and forearm by DXA. Serum levels of 25-hydroxyvitamin D were inversely correlated with BMD at the lumbar spine (p=0.012), however not related to bone mineral density at the femoral neck and forearm sites.

In summary, only two studies, to our knowledge, have examined the association of vitamin D status and bone mineral density among premenopausal women.[21, 22] These studies found positive associations between vitamin D status and bone mineral density, but did not control for important confounding factors such as calcium intake and physical activity. Therefore, we propose to examine the association between dietary vitamin D intake and serum levels of 25-hydroxyvitamin D and bone mineral density among premenopausal participants (n=271) in the cross-sectional UMass Vitamin D Status Study. We hypothesize that among the young women, dietary sources and supplements of Vitamin D, and biochemical 25-hydroxyitamin D levels will be positively associated with bone mineral content and bone area.

CHAPTER 2

METHODS

Study population

The UMass Vitamin D Status Study recruited women aged 18-30, who live in Amherst and the surrounding area. Women were recruited for this study between 2006 and 2011 by means of fliers posted around the University campus, by table tents near the dining commons, and by classroom announcements.[23]

In a single clinic visit, the study participants completed a modified version of the Harvard Food Frequency Questionnaire, as well as a comprehensive lifestyle and activity questionnaire.[23] The Harvard FFQ was modified from the 1986 version by the following ways: zucchini was included in addition to eggplant, green peppers, sauerkraut, and avocado were omitted, cole slaw and cabbage were combined into one food item, and included beets and prunes. Body mass index (BMI) and blood pressure were directly measured. Questions about reproductive health, premenstrual symptoms, attention deficit hyperactivity disorder, perceived stress and medication use were collected.[23] Before the end of the study visit, questionnaires were assessed for completeness and participants were asked to clarify any missing, unclear, or incomplete data.[24] At the time of their clinic visit, each participant provided a fasting blood sample, used to determine vitamin D.[23]

Eligible women were aged 18-30 who lived in Amherst or the surrounding area. Women were not eligible to participate in this study if they: 1) were pregnant or not currently menstruating; 2) had a history of high blood pressure or cholesterol, kidney or liver disease, bone disease (i.e. osteomalacia), gastrointestinal disorders, rheumatologic

disease, multiple sclerosis, thyroid disease or hyperparathyroidism, cancer, type 1 or 2 diabetes, or polycystic ovaries; or 3) were currently taking corticosteroids, anabolic steroids, anticonvulsants, cimetidine, or propranolol.[23]

We also excluded all participants who did not have complete data on bone mineral density and vitamin D status.

Assessment of Vitamin D

Vitamin D was measured in two ways. First, a modified version of the Harvard Food Frequency questionnaire collected the average intake of 131 foods and supplements for the two months prior to the participant's clinic visit.[24] Vitamin D-rich foods included in the questionnaire were fortified dairy foods, orange juice, breakfast cereals, dark meat, and fish.[23] Participants were also asked to specify the brand and the type of multivitamin used on the food frequency questionnaire. The Harvard FFQ was analyzed at Harvard University. Specifically, the frequency of intake of each food item was multiplied by its vitamin D content, and then summed across all items of food. Contributions from vitamin D supplemental sources were added to contributions from dietary vitamin D to calculate total vitamin D intake.[25] Intake of vitamin D foods and supplements from the FFQ were examined continuously (Table 2).

The Harvard Food Frequency Questionnaire was previously validated by Willett et al.[26, 27] Four one-week dietary records and two food frequency questionnaires were completed over one year by 173 participants. The mean correlation coefficients comparing the dietary records and first and second food frequency questionnaires were 0.44 and 0.52, respectively. The correlation coefficients of the FFQ were high and

reproducible indicating that a single FFQ is valid and reproducible for assessing specific food and beverage intake.[26, 27]

The second technique for measuring vitamin D status was via fasting blood samples, which were used to measure serum 25-hydroxyvitamin D_3 concentrations. Blood samples were processed and stored generally within two hours at -80 degrees Celsius.[28] Serum 25(OH)D_3 concentrations were determined using DiaSorin's commercially available radioimmunoassay kit.[28] Total serum 25-hydroxyvitaminD levels were examined continuously (Table 2).

The radioimmunoassay available by DiaSorin used to measure serum 25-hydroxyvitamin D_3 concentrations has been previously validated by Hollis, as well as the UMass Vitamin D Status Study research team.[28] Both within- and between-assay coefficient of variations were low (0.2-5.8%).[28]

The new recommended daily allowance is 600 IU for those aged 18-30. Thus, in the present study, Vitamin D was also categorized into intake less than 600 IU per day and intake greater than or equal to 600 IU per day for additional analyses (Table 7). Vitamin D deficiency is defined as a 25-hydroxyvitamin D level less than 50 nmol/L for those aged 18-30. In the present study, Vitamin D was also categorized into serum 25-hydroxyvitamin D levels less than 50 nmol/L and levels greater than or equal to 50 nmol/L (Table 7).

Assessment of Bone mineral density

Bone mineral content (BMC) and bone area (BA) were directly measured using what is considered the gold standard, dual-energy X-ray absorptiometry (DXA). The in

vivo precision of the specific DXA machine used, the GE Lunar Prodigy scanner, ranges from 1.0-2.2% for bone mineral content.[24] Bone mineral content, as measured in grams, provides a measure of bone mass. Bone area, as measured in cm^2, reflects a two-dimensional area, which is characterized by the periphery of a bone region.

For the UMass Vitamin D Status study, bone mineral content and bone area was measured during the participant's clinic visit using DXA scan. The total body scan mode was used on a narrow angle fan GE Lunar Prodigy Scanner (GE Lunar Corp., Madison, WI, USA).[29] Provided by the manufacturer, daily calibrations were performed using the standard calibration phantom.[24] With the exception of 31 participants, all DXA scans were performed on the morning of the participant's clinic visit.[24] All DXA scans were analyzed by a single examiner.[24]

Covariate Assessment

Information on lifestyle and demographics were collected by self-reported questionnaire at the time of the clinic visit. Questions included smoking status and number of cigarettes per day, and use of oral contraceptives and selective serotonin reuptake inhibitors (Table 1).[23] These variables were analyzed categorically (Table 1). Questions regarding physical activity were based on those previously used and validated in the Nurses Health Study II.[30] Women were asked to report the time per week that they were engaged in specific physical activities such as, walking, jogging, running, aerobics/dancing, tennis/racket sports, swimming, yoga or Pilates, and weight training.[23] Total MET-hours (i.e. Metabolic Equivalent of Task) per week was calculated for each

specific activity.[23] Physical activity in MET-hours per week was analyzed continuously (Table 1).

Each study participant's weight and height were directly measured and used to calculate BMI by the following formula: weight (kg)/height (m^2).[23] A modified version of the Harvard Food Frequency Questionnaire (FFQ) was used to collect data on participant total caloric intake, as well as dietary intake of calcium. Sun exposure was determined by self-reported time spent outdoors wearing minimal clothing, use of tanning beds, use of sunscreen, and recent travel to sunny locations.[23] The variables BMI and sun exposure were analyzed categorically, and the variables for daily intake of calories and calcium were analyzed continuously (Table 1).

Statistical Analysis

We present the distribution of vitamin D intake from dietary and supplement data as well as serum 25-hydroxyvitamin D levels (Table 2) and the distribution of bone mineral content and bone area (Table 3).

Covariate distributions were assessed against total vitamin D intake and 25(OH)D (Table 4) and against bone mineral content and bone area (Table 5). Categorical covariate variables were assessed using t-tests or analysis of variance, as appropriate. Means, standard deviations and p-values are reported. Continuous covariate variables were assessed using unadjusted linear regression, modeled as the exposure/outcome as the dependent variable and the covariate as the independent variable. Beta coefficients, standard errors, and p-values are reported.

Unadjusted linear regression models the association between vitamin D status and bone mineral density. We report beta coefficients, standard errors and p-values (Table 6). We used multivariable linear regression to examine the association between vitamin D and bone mineral content and bone area (Table 6). Covariates were assessed for confounding through individual inclusion into the age-adjusted model of vitamin D status and bone mineral content and bone area. We performed the change in estimate procedure, and covariates that resulted in a 10% change in any of the bone mineral density status were considered confounders in the final model. Physical activity in METs, calcium intake, BMI, total caloric intake were included in the final model due to their recognized associations with vitamin D and bone mineral density in the current literature.[31-34] The covariates that resulted in a 10% change in either bone mineral content or bone area and were considered confounders in the final model were age, ever smoke, and daily intakes of calcium and protein. Thus, we retained variables age, body mass index, ever smoke, physical activity in METs, caloric intake, calcium intake, and protein intake in our final multivariable models. After adjustment, vitamin D measures were not associated with bone mineral content. All analyses were performed using statistical software STATA 14.2 (StataCorp LLC).

CHAPTER 3

RESULTS

Of the 298 participants, 290 had complete dietary vitamin D data as recorded from the FFQ, 284 had serum 25(OH)D levels available, and 279 had complete bone outcome data; thus, 271 were included in the present analysis as they had complete data for our exposure and outcomes, as well as complete data recorded of our covariates. The average age of study participants was 21.4 years old. The majority, 86% of the study participants were white. As portrayed in Table 1, 79% of study participants were of underweight or normal BMI, with the remaining 21% overweight, obese category I or II.

Mean vitamin D intake and bone measures in the population are presented in tables 2 and 3. The total mean for participant self-reported vitamin D IU was 372.7 (standard deviation 285.8). Self-reported vitamin D IU for participants who took vitamin D supplement was mean value of 140.9 (standard deviation 232.3), and for participants' vitamin D from food was mean value of 231.8 (standard deviation of 182.0). Serum 25-hydroxyvitamin D levels measured in nmol/L mean value for participants was 88.0 (standard deviation of 36.7).

Among the 271 participants, the distribution of bone mineral density status is as follows. We observed a mean of 2547.6 g (standard deviation of 347.0 g) for bone mineral content, and a mean of 2190.7 cm^2 (standard deviation of 207.7 cm^2), as measured by dual energy X-ray absorptiometry.

Calories (kcal) ($P<0.001$), total fat (gm) ($P=0.006$), iron (mg) ($P<0.001$), total calcium intake (mg) ($P<0.001$), protein (gm) ($P<0.001$), and vitamin A (IU) ($P<0.001$)

measures were positively associated with total vitamin D intake (IU); however, these were not associated with serum 25(OH)D (nmol/L). In analysis of variance, BMI (kg/m^2) was associated with both bone mineral content (g) ($P<0.001$) and bone area (cm^2) ($P<0.001$). Physical activity, in MET (hours/week) was positively associated with bone mineral content (g) ($P=0.05$), but not bone area. All other covariate distributions, presented in table 4 and 5, were not statistically significant.

Total Vitamin D intake

Vitamin D intake was not associated with BMC or BA. For example, each 100 IU/day increase in total vitamin D was associated with a 4.7 g lower BMC ($P = 0.55$) Similarly, each 100 IU/day increase in total vitamin D was associated with a 0.01 cm^2 lower BA ($P=0.83$).

Total Vitamin D from food sources

Each 100 IU/day increase in vitamin D from food sources was associated with a 17.8 g lower BMC ($P = 0.23$). Each 100 IU/day increase of vitamin D from foods was associated with a 0.08 cm^2 lower BA ($P=0.41$).

Serum 25-hydroxyvitamin D

Each 10 nmol/L increase in 25(OH)D was associated with a 5.9 g higher BMC ($P=0.25$). Similarly null, each 10 nmol/L increase in 25(OH)D was associated with a 0.03 cm^2 higher BA ($P=0.36$).

Recommended levels of Vitamin D

The new recommended daily allowance is 600 IU for those aged 18-30. Thus, in the present study, Vitamin D was categorized into intake less than 600 IU per day and intake greater than or equal to 600 IU per day. The number of participants whose vitamin D intake that was greater than or equal to the recommended allowance of 600 IU per day was 45 (16.6%). A great majority of the study participants (n=226, 83.4%) had vitamin D intake that was less than the recommended allowance of 600 IU/day.

In unadjusted models, the difference between those whose vitamin D intake was less than the recommended allowance of 600 IU per day and those whose vitamin D intake was greater than or equal to the recommended allowance of 600 IU per day is 30.3 g higher BMC (P = 0.59). The difference between those whose vitamin D intake was less than the recommended allowance of 600 IU per day and those whose vitamin D intake was greater than or equal to the recommended allowance of 600 IU per day is 0.14 cm^2 higher BA (P = 0.68).

After adjustment, the difference between those whose vitamin D intake was less than the recommended allowance of 600 IU per day and those whose vitamin D intake was greater than or equal to the recommended allowance of 600 IU per day is 46.6 g lower BMC (P = 0.41). The difference between those whose vitamin D intake were less than the recommended allowance of 600 IU per day and those whose vitamin D intake was greater than or equal to the recommended allowance of 600 IU per day is 0.24 cm^2 lower BA (P=0.50).

Recommended levels of 25-hydroxyvitamin D

Vitamin D deficiency is defined as a 25-hydroxyvitamin D level less than 50 nmol/L for those aged 18-30. In the present study, Vitamin D was categorized into serum 25-hydroxyvitamin D levels less than 50 nmol/L and levels greater than or equal to 50 nmol/L.

In unadjusted models, the difference between those whose 25-hydroxyvitamin D levels less than 50 nmol/L and those whose 25-hydroxyvitamin D levels were greater than or equal to 50 nmol/L is 110.7 g higher BMC (P = 0.095). The difference between those whose 25-hydroxyvitamin D levels less than 50 nmol/L and those whose 25-hydroxyvitamin D levels were greater than or equal to 50 nmol/L is 0.73 cm^2 higher BA (P = 0.06).

After adjustment, the difference between those whose 25-hydroxyvitamin D levels less than 50 nmol/L and those whose 25-hydroxyvitamin D levels were greater than or equal to 50 nmol/L is 48.3 g higher BMC (P=0.42). The difference between those whose 25-hydroxyvitamin D levels less than 50 nmol/L and those whose 25-hydroxyvitamin D levels were greater than or equal to 50 nmol/L is 0.42 cm^2 higher BA (P = 0.25).

CHAPTER 4

DISCUSSION

Epidemiology of the relationship between vitamin D and bone mineral density

We found a null association between total vitamin D, vitamin D from foods, and serum 25-hydroxyvitamin D and both BMC and BA. Our results are somewhat consistent with previous findings of two prior studies that investigated the association between vitamin D and bone density among premenopausal women.[21, 22] The case-control study[21] found a null association, while the cross-sectional study[22] that examined the association between serum 25-hydroxyvitamin D and bone mineral density among premenopausal women found that vitamin D was positively associated with bone mineral density. Even though one of these studies adjusted for age and body mass index,[22] neither of these studies controlled for important confounding factors such as calcium intake and physical activity.[21, 22] Further, we examined the relationship between vitamin D and BMC and BA also adjusting for ever smoke, caloric intake and protein intake. This could be the reasoning why our results are not consistent with the aforementioned studies. The relationship between Vitamin D and BMC and BA may have been different in the other two studies if they had collected data on these important confounding factors, calcium intake, physical activity, ever smoke, caloric intake and protein intake.

The two studies were similar in both exposure and outcome assessment. In the cross-sectional study, Adami et al. collected serum samples of healthy premenopausal women with regular monthly cyclic menses.[22] Evaluation of bone mineral density was performed via dual-energy X-ray absorptiometry (DXA) scan at the lumbar spine and the femoral neck. In the case-control study, Olama et al. collected venous blood samples of

25-hydroxivitamin D in patients with fibromyalgia syndrome.[21] Bone mineral density was measured at the lumbar spine, femoral neck, and forearm by DXA. Thus, both studies were similar in exposure and slightly similar in outcome assessment. These previous studies looked at bone mineral density in specific regions, while ours looked at total body BMC and BA, which could further explain the different findings.

For the unadjusted results in the study conducted by Adami et al, the relationship between the logarithmic values of 25-hydroxyvitamin D concentrations and spine BMD, femoral neck BMD and total hip BMD is +0.025 (non-significant p-value >0.1), -0.074 (P=0.034), and -0.047 (non-significant p-value >0.1), respectively. In the study conducted by Olama et al., after adjustment, serum levels of 25-hydroxyvitamin D were inversely correlated with BMD at lumbar spine (p = 0.012), and null for femoral neck and forearm locations.

In the only cross-sectional study to assess 25-hydroxyvitamin D levels and bone mineral density in healthy premenopausal women, Adami et al., after adjustment for age and BMI, the regression coefficients between 25-hydroxyvitamin D concentrations and spine and femoral neck bone mineral density were +0.084 grams per centimeter squared (p=0.043) and +0.013 grams per centimeter squared (non-significant p-value), respectively.[22] Similar to the previous literature, this study did not measure self-reported dietary vitamin D values from a food frequency questionnaire.

Our study is not similar to these findings, in that Adami et al. found a positive association between serum 25-hydroxyvitamin D and both BMC and BA, while our results were null. The outcomes were different across studies in comparison to our study methods. Olama et al found a null association with a sample size of 100, whose null

results may be due to the sample size. While Adami et al found a positive association with a sample size of 608. Both of these studies had bone mineral density, measured in grams per centimeter squared as an outcome. Contrastingly, the present study used bone mineral content, measured in grams, and bone area, measured in centimeters squared. As we did not evaluate bone mineral density, but rather bone mineral content and bone area, this could explain the different findings of our null results from the current literature.

In the cross-sectional study conducted by Adami et al., the level of 25-hydroxyvitamin D where an association between serum 25-hydroxyvitamin D and bone mineral density was detectable was 20 ng/ml. In comparing this to the present study, this value is equivalent to 50 nmol/L. Our mean for 25-hydroxyvitamin D concentrations was 88.0 nmol/L, with a standard deviation of 36.7 nmol/L. Given these values, our study does not have enough power to detect an association between serum 25-hydroxyvitamin D concentrations at the low end of the range and bone density.

Non-differential misclassification of vitamin D

The Harvard Food Frequency Questionnaire was previously validated by Willett et al., indicating that a single FFQ is valid and reproducible for assessing micronutrient intake.[26] Vitamin D intake was calculated using a modified version of the Harvard Food Frequency questionnaire. However, it is possible that women incorrectly reported their intake of vitamin D-rich foods, due to a misunderstanding of what constitutes a single serving of food. To the extent that non-differential misclassification occurs, our observed association for the continuous assessment of vitamin D intake would be an underestimate of the true association, causing a bias toward the null. In the present study, the

relationship between total vitamin D and vitamin D from foods and bone mineral density and bone area suggest a null relationship. Therefore, non-differential misclassification of vitamin D on the food frequency questionnaire is possible. Further, non-differential misclassification of nutrients is common in nutritional epidemiological studies.

Vitamin D status was measured using fasting blood samples of serum 25-hydroxyvitamin D_3 concentrations. Any potential misclassification of exposure is due to lab measurement error and the resulting non-differential misclassification of serum 25-hydroxyvitamin D_3 concentrations would underestimate the true association and result in a bias toward the null. However, this is unlikely because the radioimmunoassay used to measure serum 25-hydroxyvitamin D_3 concentrations has been previously validated by UMass Vitamin D Status Study research team, and both within- and between-assay coefficient of variations were low (0.2-5.8%).[28]

Non-differential misclassification of bone mineral density

Bone mineral content and bone area were measured with the DXA scan. If the measurements of bone mineral content and bone area were incorrectly measured, then the observed association would be an underestimate of the true association. Bone mineral content and bone area was measured with what is widely accepted as the standard. Furthermore, the in vivo precision indicates that this method is valid and reproducible for assessing this outcome.[28] Therefore, we expect misclassification of outcome to be unlikely and the impact to be minor.

The range of the bone measures in the present study is as follows: for bone mineral content is 1833-3682 grams, and for bone area is 1684-2709 centimeters squared.

In the only study that had a positive association between serum 25-hydroxyvitamin D values and bone mineral density,[22] the range of bone mineral density at the spine, femoral neck, and total hip is: 1.051 ± 0.122 grams per centimeter squared, 0.919 ± 0.119 grams per centimeter squared, and 0.804±0.124 grams per centimeter squared, respectively. Our study outcomes are comparable to the study conducted by Adami et al. As determined by the range of bone mineral content and bone area, there was a wide enough range to allow us to see a relationship between serum 25-hydroxyvitamin D and bone mineral content and area.

Selection Bias

The present study is cross-sectional, and as such information is collected on exposure and outcome at the same time at baseline. Differential selection of participants into the study based on their exposure and outcome could occur if people who were both vitamin D deficient and had low bone mineral density were more motivated to participate. This would cause an increase in the "a" cell, and an over-estimate of the true association. However, selection bias is unlikely because women who participated were unaware of their bone mineral density and vitamin D status.

Information Bias

Information bias occurs when information on disease outcome is collected in a differential way between exposed and non-exposed groups. In this cross-sectional study, information bias could occur if those with low bone mineral density were more motivated to recall vitamin D intake than those with normal bone mineral density. As a result of this

recall bias, we would see an overestimate of the frequency of exposure among those with low bone mineral density, and a bias away from the null. Reducing this concern are several facts. First, the Harvard Food Frequency Questionnaire used for this study to collect vitamin D intake was previously validated by Willett et al. with high validity.[26] Second, the women enrolled into the study did not know their bone mineral density at the time of their clinic visit. Third, the food frequency questionnaire collected a wide variety of information such as calcium, alcohol and other nutrients; thus, the participants were unaware of hypothesis at the time of their visit. Therefore, we believe that recall bias in this study is unlikely and the impact would be minor.

Confounding

Confounders are other risk factors for the outcome of interest (i.e. low bone mineral density) and are also associated with the exposure of interest. However, it is likely that residual confounding will remain after creation of the final model because not all covariates are perfectly measured. In addition, we do not have participant information on diseases such as the autoimmune disease, systemic lupus erythematosus. Women who have systemic lupus erythematosus may spend less time in the sun, and thus receive less vitamin D. As such, if systemic lupus erythematosus was negatively associated with both vitamin D status[35] and bone mineral density,[36] we would observe an overestimate of the true association. However, lupus is an uncommon disease, and therefore we would not expect this to be a confounder.

In our study, we analyzed characteristics other than vitamin D intake of the exposed group that could have led to their developing low bone mineral density. We

collected information on potential confounders, including physical activity (measured as total MET-hrs/week), sun exposure, calcium intake, total caloric intake, selective serotonin reuptake inhibitor use, and oral contraceptive use. To reduce confounding of the association between vitamin D and bone mineral density by these variables, we used multivariable linear regression.

Temporal Bias

Cross-sectional studies are subject to temporal bias, an inability to determine whether the exposure preceded the outcome. If bone mineral density influences exposure status (i.e. vitamin D), then we cannot conclude that the intake of vitamin D intake preceded the status of bone mineral density. We would therefore conclude that the observed association incorrectly describes the association between vitamin D intake and bone mineral density. However, it is unlikely that bone mineral density influences the status of vitamin D because the proposed physiological mechanism of this association is that vitamin D_3 assists in bone mineralization. Therefore, since vitamin D_3 is responsible for the absorption of calcium into bone from the small intestine, the threat of temporal bias is minimized.

Generalizability

One of the proposed physiological pathways for the association between vitamin D intake and bone mineral density is that Vitamin D_3 assists in bone mineralization, as it is involved in the activation of osteoblasts and osteoclasts. Vitamin D_3 is responsible for the absorption of calcium into bone from the small intestine. There is no evidence to

suggest this association would be different among other women not included in the study, therefore the results should be generalizable to most people. If the physiological mechanism for the association between vitamin D intake and bone mineral density is different among men, then the observed association could not be generalized to men.

CHAPTER 5

CONCLUSION

In our study, vitamin D was not associated bone mineral density outcomes. Our results indicated a null association between vitamin D and BMC and BA, a null association between vitamin D from foods and BMC and BA, and a null association between serum 25-hydroxyvitamin D and BMC and BA. The previous study conducted, which found a positive relationship between serum 25-hydroxyvitamin D and bone mineral density, was substantially larger.[17] Further studies with larger sample sizes as well as with daily FFQ recordings, are warranted to make a public health recommendation of peak bone mass with vitamin D intake.

Table 1: Characteristics of Participants; UMass Vitamin D Status Study, 2006-2011, N=271

	N (%)	Mean (SD)
Age (years)		21.4 (2.8)
Physical activity (MET hours/week)		54.7 (48.7)
Race		
White	233 (86.3)	
Other	37 (13.7)	
Body Mass Index (BMI), kg/m2		
		22.9 (3.2)
Underweight (<18.5)/Normal (18.5-24.9)	214 (79.0)	
Overweight (25.0-29.9)/Obese I (30-34.9) / Obese II (35-39.9)	57 (21.0)	
Regular sunscreen use		
Yes	158 (58.3)	
No	113 (41.7)	
Current medication use		
Selective serotonin reuptake inhibitors	12 (4.8)	
Other anti-depressants	3 (1.2)	
Antacids	6 (2.4)	
Other	58 (23.0)	
Curent oral contraceptive user		
Non- user	154 (56.8)	
Curent user	117 (43.2)	
Smoking		
Never	230 (84.9)	
Ever	41 (15.1)	
Calories (kcal/day)		2197.4 (889.4)
Total fat (gm/day)		72.3 (33.5)
Iron (mg/day)		23.6 (15.4)
Total calcium intake (mg/day)		1220.3 (618.5)
Protein (gm/day)		97.8 (46.3)
Vitamin A (IU/day)		17345.4 (11041.2)

Table 2. Mean dietary intake and vitamin D status, UMass Vitamin D Status Study, 2006-2011, N=271

	Mean (SD)
FFQ Vitamin D (IU)	372.7 (285.8)
Vitamin D from supplements	140.9 (232.3)
Vitamin D from food	231.8 (182.0)
Total Serum 25(OH)D (nmol/L)	88.0 (36.7)

Table 3. Distribution of Bone mineral content and Bone area, UMass Vitamin D Status Study, 2006-2011, N=271

	Mean (SD)	Derivation
Bone mineral content, g	2547.6 (347.0)	Measured by DXA
Bone area, cm^2	2190.7 (207.7)	Measured by DXA

Table 4: Covariate distributions by daily total Vitamin D intake and 25(OH)D levels; UMass Vitamin D Status Study, 2006-2011

	Total vitamin D intake (IU)			Serum 25(OH)D (nmol/L)		
	Mean	**SD**	**p-value**	**Mean**	**SD**	**p-value**
Race*						
White	380.4	285.8		89.5	36.1	
Other	332.4	285.6		78.9	39.9	
			0.34			0.11
Body Mass Index (BMI), kg/m2						
Underweight (<18.5)/Normal (18.5-24.9)	364.9	277.2		86.4	35.6	
Overweight (25.0-29.9)/Obese I (30-34.9)/	401.7	317.2		94.3	40.3	
Obese II (35-39.9)			0.39			0.14
Regular sunscreen use						
Yes	363.8	297.9		86.8	34.3	
No	385.1	268.8		89.8	39.9	
			0.55			0.51
Current medication use						
Not taking any medications	354.6	283.9		87.5	37.4	
Antidepressant use	335.2	216.9		80.6	31.4	
			0.79			0.48
Curent oral contraceptive user						
Non- user	368.1	305.9		84.6	36.2	
Curent user	378.7	258.1		92.6	37	
			0.76			0.07
Smoking						
Never	381.2	286.3		87.4	35.8	
Ever	324.8	281.6		91.6	41.7	
			0.24			0.49

	β	**SE**	**p-value**	**β**	**SE**	**p-value**
	-0.37	7.4	0.96	-2.96	4.5	0.5
Age, in years	0.83	0.43	0.05	0.22	0.26	0.39
Physical activity, in MET (hours/week)	0.04	0.024	0.08	-0.01	0.01	0.2
Calories (kcal/day)	0.61	0.63	0.34	0.13	0.38	0.73
Total fat (gm/day)	1.3	1.37	0.34	0.94	0.82	0.25
Iron (mg/day)	6.07	0.03	0.09	0.03	0.02	0.82
Total calcium intake (mg/day)	0.17	0.46	0.72	-0.03	0.27	0.92
Protein (gm/day)	0.003	0.36	0.17	0.001	0.001	0.18

Table 5: Covariate distributions vs. Bone mineral content and bone area; UMass Vitamin D Status Study, 2006-2011

	Bone mineral content (g)			Bone area (cm^2)		
	Mean	**SD**	**p-value**	**Mean**	**SD**	**p-value**
Race*						
White	2551.9	334.8		2196.9	202.3	
Other	2530.2	419.9		2158.5	238.8	
			0.72			0.3
Body Mass Index (BMI), kg/m2						
Underweight (<18.5)/Normal (18.5-24.9)	2483.2	314.2		2156.8	193.8	
Overweight (25.0-29.9)/Obese I (30-34.9)/	2789.5	359.7		2317.98	210.4	
Obese II (35-39.9)			<0.001			<0.001
Regular sunscreen use						
Yes	2551.4	353.9		2191.3	208.5	
No	2542.3	338.5		2189.9	207.5	
			0.83			0.96
Current medication use						
Not taking any medications	2553	367.6		2188.1	217.5	
Antidepressant use	2533.3	273.5		2192.3	180.7	
			0.84			0.94
Curent oral contraceptive user						
Non- user	2542.7	366.5		2188.4	205.6	
Curent user	2554.1	320.9		2193.8	211.2	
			0.79			0.83
Smoking						
Never	2560.8	352.7		2198.2	210.4	
Ever	2473.7	306.4		2149	189.1	
			0.14			0.16

	β	SE	p-value	β	SE	p-value
Age, in years	-0.37	7.4	0.96	-2.96	4.5	0.5
Physical activity, in MET (hours/week)	0.83	0.43	0.05	0.22	0.26	0.39
Calories (kcal)	0.04	0.024	0.08	0.02	0.01	0.2
Total fat (gm)	0.61	0.63	0.34	0.13	0.38	0.73
Iron (mg)	1.3	1.37	0.34	0.94	0.82	0.25
Total calcium intake (mg)	0.06	0.03	0.09	0.03	0.02	0.21
Protein (gm)	0.17	0.46	0.72	-0.03	0.27	0.92
Vitamin A (IU)	0.003	(0.002)	0.17	0.001	0.001	0.18

Table 6: Association of daily vitamin D intake and 25(OH)D levels with bone mass; UMass Vitamin D Status Study, 2006-2011

	Bone mineral content (g)			Bone area (per 100 cm^2)		
	β	SE	p-value	β	SE	p-value
Total vitamin D (per 100 IU/day)						
Unadjusted	4.7	7.4	0.52	0.03	0.04	0.47
Adjusted*	-4.7	7.9	0.55	-0.01	0.05	0.83
Vitamin D from foods only (per 100 IU/day)						
Unadjusted	-8.4	11.6	0.47	-0.05	0.07	0.49
Adjusted*	-17.8	14.9	0.23	-0.08	0.09	0.41
Serum 25OHD (per 10 nmol/L)						
Unadjusted	7.99	5.7	0.16	0.04	0.03	0.22
Adjusted*	5.9	5.1	0.25	0.03	0.03	0.36

*Adjusted for age (continuous, year), BMI (continuous, kg/m^2), ever smoke (categorical, Yes/No), physical activity (continuous, MET hours/week), and daily intake of calories (continuous, kcal/day), calcium (continuous, mg/day) and protein (continuous, gm/day)

Table 7: Association of daily vitamin D intake at the RDA level and 25(OH)D at level of deficiency with bone mass; UMass Vitamin D Status Study, 2006-2011

	Bone mineral content (g)			Bone area (per 100 cm^2)		
	β	SE	p-value	β	SE	p-value
Total vitamin D <600 IU vs. ≥600 IU						
Unadjusted	30.3	56.7	0.59	0.14	0.34	0.68
Adjusted*	-46.6	56.3	0.41	-0.24	0.35	0.5
Serum 25OHD <50 nmol/L vs. ≥50 nmol/L						
Unadjusted	110.7	65.99	0.095	0.73	0.39	0.06
Adjusted*	48.3	59.5	0.42	0.42	0.37	0.25

*Adjusted for age (continuous, year), BMI (continuous, kg/m^2), ever smoke (categorical, Yes/No), physical activity (continuous, MET hours/week), and daily intake of calories (continuous, kcal/day), calcium (continuous, mg/day) and protein (continuous, gm/day)

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