ARTICLE

Vitamin D, Calcium, and Dairy Intakes and Stress Fractures Among Female Adolescents

Kendrin R. Sonneville, ScD, RD; Catherine M. Gordon, MD, MSc; Mininder S. Kocher, MD, MPH; Laura M. Pierce, BA; Arun Ramappa, MD; Alison E. Field, ScD

Objective: To identify whether calcium, vitamin D, and/or dairy intakes are prospectively associated with stress fracture risk among female adolescents.

Design: Prospective cohort study.

Setting: Adolescent girls living throughout the United States.

Participants: A total of 6712 girls aged 9 to 15 years at baseline in the Growing Up Today Study, an ongoing prospective cohort study.

Main Exposures: Dairy, calcium, and vitamin D intakes assessed by food frequency questionnaire every 12 to 24 months between 1996 and 2001.

Main Outcome Measure: Incident stress fracture that occurred between 1997 and 2004 as reported by mothers of the participants in 2004. Cox proportional hazards models were used to examine associations.

Results: During 7 years of follow-up, 3.9% of the girls developed a stress fracture. Dairy and calcium intakes were unrelated to risk of developing a stress fracture. However, vitamin D intake was inversely related to stress fracture risk. The multivariable-adjusted hazard ratio of stress fracture for the highest vs the lowest quintile of vitamin D intake was 0.49 (95% CI, 0.24-1.01; P_{trend}=.07). We conducted a stratified analysis to estimate the association between vitamin D intake and stress fracture risk among girls participating in at least 1 h/d of high-impact activity, among whom 90.0% of the stress fractures occurred, and found that higher vitamin D intake predicted significantly lower risk of stress fracture (P_{trend}=.04).

Conclusions: Vitamin D intake is associated with lower stress fracture risk among adolescent girls who engage in high levels of high-impact activity. Neither calcium intake nor dairy intake was prospectively associated with stress fracture risk.

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Author Affiliations: Division of Adolescent/Young Adult Medicine, Department of Medicine (Drs Sonneville, Gordon, and Field and Ms Pierce) and Departments of Endocrinology (Dr Gordon) and Orthopedic Surgery (Dr Kocher), Children's Hospital Boston, and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital (Dr Field), Harvard Medical School, and Department of Epidemiology, Harvard School of Public Health (Dr Field), Harvard University, and Division of Sports Medicine, Department of Orthopedic Surgery, Beth Israel Deaconess Medical Center (Dr Ramappa), Boston, Massachusetts.

As participation in organized sports and athletic specialization among children and adolescents have increased, so too has the recognition of overuse injuries.[1,2] Stress fractures, which occur when stresses on bone exceed the bone's capacity to withstand and heal from those forces, are a particularly common type of injury seen in both competitive and recreational athletes.[3] We previously reported that nearly 4% of the adolescent and young adult girls in our cohort developed a stress fracture during 7 years of follow-up.[4]

See also page 684

The risk of stress fracture is influenced by extrinsic (training regimen, type of sport), intrinsic (sex, race/ethnicity), biomechanical, anatomical, and hormonal factors.[3] Nutritional intakes, particularly calcium, which is needed for bone mineralization, and vitamin D, which is needed for maintaining calcium homeostasis and bone remodeling, have been suggested as protective against stress fractures.[3] Although consuming calcium and calcium-rich dairy products is routinely encouraged for optimal bone health, the evidence for this recommendation has been challenged.[5,6] Further, while vitamin D deficiency is relatively common among adolescents,[7,8] data are lacking on the role of vitamin D intake, whether sufficient or in excess of recommended intake,[9] on bone health.[10,11]

Adolescence is the most critical period for bone mineral accrual[12-16] and therefore is considered an important window for the prevention of long-term consequences of low bone mineral content such as postmenopausal osteoporosis. The relationship between dietary intake during adolescence and short-term consequences of low bone mineral content, however, is understudied. In a cross-sectional analysis of adolescent girls, intakes of dairy, calcium, and vitamin D were all unrelated to stress fractures after controlling for age.[17] Because bone mineral is accrued over time, however, the contribution of long-term dietary exposure on stress fracture risk cannot be examined in cross-sectional studies or studies of short duration.

Physical activity is the primary modifiable stimulus for increased bone growth and development in adolescents,[5] and weight-bearing activity during childhood and adolescence seems to be a more important factor for peak bone mass than dietary intake.[18] Despite known benefits of physical activity on bone mineral content,[19] there is a threshold over which the risk of stress fracture increases significantly among adolescent girls.[17] The combined effects of diet and exercise on bone health are still unknown. More research is needed to explore whether protective dietary factors could mitigate the risk of stress fracture among adolescents who regularly engage in high-impact activities.

Stress fracture is a source of significant morbidity among female athletes during adolescence.[17] However, few studies have identified modifiable risk factors for stress fracture among female adolescents, other than participation in high-impact sports.[4,17] As such, prospective studies are needed to identify other modifiable risk factors for stress fracture among this population. The aim of this study was to identify dietary factors that are prospectively associated with risk of stress fracture among female adolescents and, in particular, those who are at highest risk for stress fracture.

METHODS

Participants were members of the Growing Up Today Study (GUTS), an ongoing cohort study of adolescents throughout the United States that was established in 1996. Participants in GUTS are the offspring of women in the Nurses' Health Study.[20] Mothers of children aged between 9 and 14 years in 1996 were sent a detailed letter that outlined the purposes of GUTS and were asked to provide parental consent for their children to enroll. Of those invited, 68.2% of their daughters (n = 9039) returned completed questionnaires, thereby assenting to participate in the cohort. Additional details of enrollment are reported elsewhere.[21] The Human Subjects Committee at Brigham and Women's Hospital approved GUTS. This analysis was approved by that committee and by the Committee on Clinical Investigation at Children's Hospital Boston.

EXPOSURES

Dietary intake, including intake of dairy foods and soda, was assessed annually from 1996 through 1999 and again in 2001 using the previously validated Youth/Adolescent Questionnaire.[22,23] The Youth/Adolescent Questionnaire is a self-administered, semiquantitative food frequency questionnaire that assesses intake during the past year using portion sizes for foods that are appropriate for each age as determined from analyses of national nutrition data.[22,23] The development, reproducibility, and validation of the Youth/Adolescent Questionnaire have been previously described.[22,23] Briefly, participants were asked how frequently they used a typical portion size of specified foods on average during the past year. Nutrient intakes were computed by multiplying the frequency of consumption of each unit of food by the nutrient content of the specified portions based on the nutrient values in foods obtained from US Department of Agriculture sources and food manufacturers; intakes include calcium or vitamin D in foods routinely fortified with these nutrients.[24]

Participants also reported calcium supplement and multivitamin use, physical activity, menstrual status, weight, and height annually from 1996 through 2001. We calculated body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) from self-reported weight and height information. Despite concern that self-reported weight and thus BMI are biased, a strong correlation between BMI calculated from self-reported vs measured height and weight has been observed in adolescent samples.[25,26]

The total number of hours of physical activity per week was assessed by asking participants to report the number of hours they spent doing 18 different types of physical activity in each of the 4 seasons. Reported physical activity that exceeded 40 h/wk was considered implausible and was not used in the analysis. High-impact activity was computed as the sum of the average hours per week a participant reported engaging in basketball, running, soccer, tennis, cheerleading, or volleyball.[17] Girls who reported participating in an average of at least 1 h/d of high-impact activity were defined as having high levels of high-impact activity.

On each questionnaire, girls were asked whether their menstrual periods had started and, if yes, how old they were when their periods began. Girls were classified as having a family history of low bone density or osteoporosis if their mothers indicated having a history of low bone density or osteoporosis on the 2005 Nurses' Health Study questionnaire.

OUTCOME

Incident stress fracture was the primary outcome. Stress fractures were reported in 2004 by the participants' mothers, who were registered female nurses participating in the Nurses' Health Study II. Mothers were asked whether a doctor had ever said that their child had a stress fracture and were asked to report the age at which the stress fracture developed and the site (foot, arm, leg, wrist, or other) of the stress fracture.

SAMPLE FOR ANALYSIS

Cases that occurred prior to enrollment in GUTS in fall 1996 and reports of a stress fracture without an age at diagnosis were excluded. A total of 6712 girls whose mothers responded to the question about stress fractures were included in the analysis.

STATISTICAL ANALYSIS

Cox proportional hazards models were used for all multivariable analyses. We modeled calcium and vitamin D intakes in 2 ways: (1) calcium and vitamin D from dietary intake only, and (2) calcium and vitamin D from both dietary and supplement intakes. Calcium and vitamin D intakes were modeled as quintiles, with the lowest quintile serving as the reference. Dairy intake was modeled as servings per day (0, 1, 2, and ≥3 servings/d), with 0 servings as the reference.

All models controlled for age. Fully adjusted models additionally controlled for known predictors of bone mineral content or stress fracture in children, including BMI, age at menarche, physical activity, and family history.[5,13] Models assessing the association with quintiles of calcium intake additionally ad-

Table 1. Baseline Demographic and Lifestyle Characteristics of 6712 Preadolescent, Adolescent, and Young Adult Girls in the Growing Up Today Study

Characteristic	Value
Age, mean (SD), y	11.6 (1.6)
White, %	93.6
BMI, mean (SD)	19.0 (3.3)
Overweight or obese, %	19.8
Underweight, %	8.6
Age at menarche, mean (SD), y	12.3 (1.2)
Maternal history of low bone density or osteoporosis, %	10.4
Nutritional intake, mean (SD)	
Calcium, mg/d	1181.5 (478.1)
Vitamin D, IU/d	376.2 (183.0)
Dairy, servings/d	2.0 (1.3)
High-impact activity, mean (SD), h/wk[a]	5.2 (4.1)

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).
[a] Sum of basketball, running, soccer, tennis, cheerleading, and volleyball.

justed for vitamin D intake (as a continuous variable centered at the mean), and models assessing the association with quintiles of vitamin D intake additionally adjusted for calcium intake (as a continuous variable centered at the mean). In secondary analyses we further controlled for soda intake, which has been suggested as a potential confounder of a calcium–bone health association.

Because we have previously shown that high levels of high-impact activity increase the risk of developing a stress fracture,[4] we investigated whether associations with dietary intake were modified by activity level. In these analyses, we stratified by level of high-impact activity (<1 h/d of high-impact activity vs ≥1 h/d of high-impact activity).

We report hazard ratios (HRs) and 95% CIs using the lowest intake category as the reference group as well as the P_{trend}. All analyses were conducted with SAS version 9.1 statistical software (SAS Institute, Inc).

RESULTS

Among the 6712 girls, 8.6% were underweight and 19.8% were overweight or obese (**Table 1**). Approximately 30.4% of the girls engaged in at least 1 h/d of high-impact activity. The majority of the participants (93.6%) were white, and 10.4% had a mother with a history of low bone density or osteoporosis. The girls consumed a mean (SD) of 2.0 (1.3) servings of dairy foods per day. At baseline, the mean (SD) intakes of calcium (1181.5 [478.1] mg/d) and vitamin D (376.2 [183.0] IU/d) were below the Recommended Dietary Allowance for girls aged between 9 and 18 years (1300 mg/d and 600 IU/d, respectively). As expected, dairy intake was strongly correlated with calcium intake ($r=0.90$; $P<.001$) and vitamin D intake ($r=0.79$; $P<.001$). Calcium and vitamin D intakes were also highly correlated ($r=0.84$; $P<.001$).

During 7 years of follow-up, 3.9% of girls developed a stress fracture. Most (90.0%) of the stress fractures occurred in girls who were participating in at least 1 h/d of high-impact activity. There was no evidence of a protective association between dairy intake and stress fracture risk. Girls consuming 3 or more servings/d of dairy were no more likely than their peers consuming no dairy to develop a stress fracture (HR=1.02; 95% CI, 0.65-1.61) (**Table 2**). Similarly, there was no suggestion that higher calcium intake from both dietary sources and supplements was protective against stress fractures. Girls in the highest quintile were no less likely than girls in the lowest quintile to develop a stress fracture (HR=1.57; 95% CI, 0.77-3.17).

Table 2. Age-Adjusted and Fully Adjusted Associations of Dairy, Vitamin D, and Calcium Intakes With Stress Fracture Among All Girls in the Growing Up Today Study

	HR (95% CI)	
Intake	Age-Adjusted	Fully Adjusted[a]
Dairy		
Servings/d		
0	1 [Reference]	1 [Reference]
1	1.28 (0.87-1.89)	1.21 (0.81-1.81)
2	0.98 (0.64-1.52)	0.91 (0.58-1.43)
≥3	1.13 (0.72-1.75)	1.02 (0.65-1.61)
P_{trend}	.83	.82
Vitamin D		
Quintile[b]		
1	1 [Reference]	1 [Reference]
2	0.93 (0.59-1.49)	0.95 (0.59-1.55)
3	0.86 (0.53-1.38)	0.72 (0.42-1.24)
4	0.92 (0.58-1.47)	0.78 (0.44-1.39)
5	0.72 (0.44-1.19)	0.49 (0.24-1.01)
P_{trend}	.25	.07
Calcium		
Quintile[c]		
1	1 [Reference]	1 [Reference]
2	0.93 (0.56-1.54)	0.94 (0.55-1.61)
3	1.12 (0.69-1.83)	1.28 (0.74-2.22)
4	0.87 (0.52-1.44)	0.99 (0.54-1.81)
5	1.22 (0.76-1.94)	1.57 (0.77-3.17)
P_{trend}	.52	.29

Abbreviation: HR, hazard ratio.
[a] Adjusted for age, body mass index, age at menarche, maternal history of low bone density or osteoporosis, and level of high-impact physical activity. Vitamin D model is additionally adjusted for mean-centered calcium intake. Calcium model is additionally adjusted for mean-centered vitamin D intake.
[b] The mean (SD) vitamin D intakes for the quintiles are as follows: quintile 1, 107 (46) IU/d; quintile 2, 210 (56) IU/d; quintile 3, 324 (43) IU/d; quintile 4, 433 (55) IU/d; and quintile 5, 663 (149) IU/d.
[c] The mean (SD) calcium intakes for the quintiles are as follows: quintile 1, 541 (124) mg/d; quintile 2, 825 (86) mg/d; quintile 3, 1111 (93) mg/d; quintile 4, 1398 (93) mg/d; and quintile 5, 1891 (298) mg/d.

Although there did not appear to be an association between vitamin D intake and fracture risk in age-adjusted models, after adjusting for confounders there was a suggestion that vitamin D intake from both dietary sources and supplements was protective against stress fractures. Girls in the highest quintile of vitamin D intake had a 50% lower risk of stress fracture compared with girls in the lowest quintile (HR=0.49; 95% CI, 0.24-1.01; P_{trend}=.07).

In an analysis stratified by level of high-impact activity, vitamin D intake was associated with a lower risk of incident stress fracture among girls who participate in at least 1 h/d of high-impact activity (**Table 3**). Among these highly active participants, girls in the highest quintile of vitamin D intake had a 52% lower risk of stress

Table 3. Fully Adjusted Associations of Dairy, Vitamin D, and Calcium Intakes With Stress Fracture Stratified by Level of High-Impact Activity Among All Girls in the Growing Up Today Study

	Fully Adjusted HR (95% CI)[a]	
Intake	High-Impact Activity <1 h/d	High-Impact Activity ≥1 h/d
Dairy		
Servings/d		
0	1 [Reference]	1 [Reference]
1	0.54 (0.15-1.97)	1.35 (0.88-2.07)
2	0.85 (0.26-2.78)	0.94 (0.57-1.53)
≥3	0.29 (0.04-2.37)	1.15 (0.71-1.84)
P_{trend}	.28	.90
Vitamin D		
Quintile[b]		
1	1 [Reference]	1 [Reference]
2	0.45 (0.08-2.36)	1.04 (0.62-1.73)
3	0.66 (0.11-3.93)	0.74 (0.41-1.33)
4	2.24 (0.56-8.98)	0.71 (0.38-1.32)
5	0.72 (0.07-7.99)	0.48 (0.22-1.02)
P_{trend}	.55	.04
Calcium		
Quintile[c]		
1	1 [Reference]	1 [Reference]
2	0.15 (0.02-1.02)	1.29 (0.71-2.37)
3	0.15 (0.02-1.28)	1.79 (0.97-3.30)
4	0.48 (0.10-2.25)	1.24 (0.62-2.49)
5	0.22 (0.01-3.83)	2.14 (0.98-4.69)
P_{trend}	.20	.11

Abbreviation: HR, hazard ratio.
[a] Adjusted for age, body mass index, age at menarche, maternal history of low bone density or osteoporosis, and level of high-impact physical activity. Vitamin D model is additionally adjusted for mean-centered calcium intake. Calcium model is additionally adjusted for mean-centered vitamin D intake.
[b] The mean (SD) vitamin D intakes for the quintiles are as follows: quintile 1, 107 (46) IU/d; quintile 2, 210 (56) IU/d; quintile 3, 324 (43) IU/d; quintile 4, 433 (55) IU/d; and quintile 5, 663 (149) IU/d.
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fracture compared with girls in the lowest quintile (HR=0.48; 95% CI, 0.22-1.02; P_{trend}=.04). High dairy intake was unrelated to stress fracture risk among the highly active girls (HR=1.15; 95% CI, 0.71-1.84), whereas highly active girls with high calcium intake were at increased risk for fracture (HR=2.14; 95% CI, 0.98-4.69).

When the analyses were further adjusted for soda intake, the results were virtually unchanged (data not shown). Moreover, when the analyses were restricted to calcium and vitamin D intakes from foods only (ie, not from supplement use), the results were not different from the findings reported for models including calcium and vitamin D intakes from both dietary sources and supplements.

COMMENT

We found that among 6712 preadolescent and adolescent girls living throughout the United States, vitamin D intake was predictive of a lower risk of developing a stress fracture, particularly among those who participated in at least 1 h/d of high-impact activity and thus were at increased risk for fracture. In contrast, there was no evidence that calcium and dairy intakes were protective against developing a stress fracture or that soda intake was predictive of an increased risk of stress fracture or confounded the association between dairy, calcium, or vitamin D intakes and fracture risk. In a stratified analysis, we observed that high calcium intake was associated with a greater risk of developing a stress fracture. This unexpected finding warrants further inquiry.

Our findings support the Institute of Medicine's recent increase in the Recommended Dietary Allowance for vitamin D for adolescents from 400 IU/d to 600 IU/d.[9] We observed a linear trend for lower stress fracture risk with increasing vitamin D intake such that those in the highest quintile had about 50% the risk of developing a stress fracture compared with those in the lowest quintile. Because too few participants had a vitamin D intake higher than 600 IU/d, we were unable to explore the potential benefits of vitamin D intake in excess of the Recommended Dietary Allowance. Moreover, at the time the data were collected, vitamin D supplements were uncommon; thus, we were unable to assess whether intake in excess of 800 to 1000 IU/d, which has been recommended by some experts,[27-29] lowered the risk of stress fracture. Future research is needed to ascertain whether the risk of stress fracture can be lowered further with higher doses of vitamin D.

To our knowledge, no previous longitudinal studies have examined the influence of dietary intake on the risk of developing a stress fracture among a general population of female adolescents. Thus, it is challenging to compare our results with those of prior studies, many of which have focused on specific high-risk populations such as competitive athletes. Our findings are not consistent with those reported in a prospective study of 125 young adult female competitive distance runners, which found that higher intakes of calcium and dairy products predicted lower rates of stress fracture and that higher intakes of vitamin D, calcium, and dairy foods were all associated with significant gains in hip bone mineral density during the 2 years of follow-up.[30] The discrepancy in results could be because of the fact that our sample included preadolescents and adolescents, which are critical windows in bone development. Our findings are supported by related evidence from genomic analyses,[31] which have reported a relationship between vitamin D receptor gene polymorphisms and bone mineral density in a sample of adolescent girls[32] and risk of stress fracture in an adult sample.[33]

Our findings are supported by several studies that have found no association between dairy intake and bone health in children and adolescents.[5,6] While 2 randomized trials reported a positive relationship between dairy product consumption and measures of bone health,[34,35] most cross-sectional, retrospective, and prospective studies did not.[36-42] A review of calcium intake, dairy product intake, and bone health found that the vast majority of controlled studies of dairy supplementation or total dietary calcium intake show that, although very low calcium intake may be harmful to bone development, increases in dairy or total dietary calcium intakes higher than 400 to 500 mg/d are not correlated with nor represent a predictor of bone mineral density or fracture rate in children or adolescents.[5] This review concluded that there is no empirical evidence for the recommendation to increase dairy intake in children and adolescents to promote bone mineralization.[5] One cross-sectional study of bone mass among pubertal girls in Beijing, China, however, found a significant correlation between milk consumption and bone mineral content and reported that vitamin D was the most important nutrient in milk.[43] Further, low vitamin D status was associated with lower bone mineral density of the forearm in a study of 12- and 15-year-old girls.[44]

This study is subject to several limitations. Although the participants in the GUTS cohort reside across the United States, the sample is not representative of the US population, therefore potentially limiting the generalizability of our findings. Because the participants are children of registered nurses, female subjects of low socioeconomic status are underrepresented. Our sample lacks the racial/ethnic diversity needed to further explore racial differences in stress fracture risk, which one would expect given the known racial difference in calcium retention and bone health.[45-48] Finally, GUTS participants have high levels of physical activity and thus may be a select sample of youth. Although the prevalence of stress fractures could have been overestimated because cases were reported by mothers, the misclassification would likely bias the results toward the null; thus, we may have presented conservative estimates. We report an association between vitamin D intake, not vitamin D status, of our participants and risk of stress fracture. Because vitamin D status is only partly dependent on dietary intake of vitamin D,[49] the association between vitamin D status and stress fracture risk should be explored in future studies to help elucidate the mechanism through which vitamin D may alter stress fracture risk. The strengths of our study, including its prospective design, geographically diverse sample, validated measures, and large sample size, far outweigh the limitations. Moreover, characteristics of our sample that limit generalizability enhance our ability to study stress fractures because white participants and those with high levels of physical activity are at highest risk for developing this type of fracture. Future studies are needed to distinguish stress fractures from other overuse injuries and, if possible, to include radiographic confirmation of the fractures.

Stress fracture is a frequent cause of injury in recreational athletes, with consequences ranging from delayed return to full sport participation and reoccurrence of injury.[50] Given the limited knowledge of modifiable risk factors for stress fracture among adolescent girls, the results of this study provide important information regarding the role of dietary factors in the prevention of stress fracture. Among the dietary factors studied, only vitamin D intake appeared to be related to lower risk of stress fracture among female adolescents. The protective association was seen among girls at increased risk for stress fracture due to their high activity levels. Future studies are needed to ascertain whether vitamin D intake from supplements confers a similarly protective effect as vitamin D consumed through dietary intake.

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Correspondence: Kendrin R. Sonneville, ScD, RD, Division of Adolescent/Young Adult Medicine, Children's Hospital Boston, 300 Longwood Ave, AU-514, Boston, MA 02115 (kendrin.sonneville@childrens.harvard.edu).
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