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The Association between Milk Intake and Vitamin D Status in Adolescent Girls: Research Article

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Abstract:

Background: Sufficient vitamin D and calcium intake are essential for optimal bone health but vitamin D is gaining attention as being linked to other health benefits. The purpose of this analysis was to evaluate the relationship between milk intake and serum vitamin D levels in a cohort of adolescent girls.

Methods: A cross-sectional analysis of 124 adolescent girls was done using t-tests to compare the differences between the control and dairy groups and multiple linear regression was used to investigate predictors of serum vitamin D.

Results: More than half girls (58%) had deficient vitamin D levels and nearly 20% were considered insufficient. Within the overall regression model, milk intake was not statistically significant in predicting serum 25(OH)D, however, Body Mass Index (BMI), race, and season were all statistically significant predictors.

Conclusion: In this cohort, as in other studies, milk drinking was not associated with higher serum 25(OH)D levels in adolescent girls. For adolescents to achieve adequate serum levels and health benefits, providers need to evaluate individual vitamin D levels. For those with low levels, additional education and/or supplementation may be needed.

Introduction: The importance of vitamin D in health promotion and disease prevention has gained wide recognition in recent years. It is well accepted that sufficient vitamin D is essential for optimal bone health. Vitamin D, along with dietary calcium, is especially important during adolescence when individuals accrue approximately 45% of their adult bone mass, an important determinant of osteoporotic fracture later in life. Although the role of vitamin D in body systems has not been fully elucidated, vitamin D has been linked to the prevention of many diseases such as cancer, metabolic syndrome, diabetes, and hypertension [1-4]. In children and adolescents, vitamin D is associated with diabetes, systolic blood pressure, and high density lipoprotein [5]. Thus, vitamin D status of adolescents is an important public health consideration.

Estimates are that up to 70% of U.S. children and adolescents are vitamin D deficient or insufficient, based on serum 25 hydroxyvitamin D (25[OH]D), the functional indicator of vitamin D status [6-8]. Vitamin D deficiency has been defined by the Institute of Medicine (IOM), the American Academy of Pediatrics (AAP) and the Endocrine Society (ES) as a serum 25(OH)D of <20 ng/ml while insufficiency is defined as 25(OH)D of 21-29 ng/ml [9-12]. However, the ES guidelines set a higher cut-off of < 30 ng/mL in order to achieve the additional health benefits [12]. The IOM and AAP define the upper limit of 101-149 ng/ml as excess 25(OH)D. The ES does not define an upper limit. Risk factors for lower serum 25(OH)D in youth are geographical location, lack of sunlight exposure, sunscreen use, darker skin and being overweight [9,13].

Vitamin D is an essential hormone that can be obtained from cutaneous biosynthesis and dietary intake or supplementation [14]. It is increasingly difficult to obtain adequate levels of vitamin D from sunlight because of more time spent indoors and the use of sunscreen during outdoor activity. Sunscreen blocks most of the vitamin D conversion in the skin. Therefore, dietary intake is an important source of vitamin D. The IOM set 600 international units (IU) daily as the recommended dietary allowance (RDA) for adolescents [15]. Since there are few food sources of vitamin D, a conscious effort is needed to obtain adequate vitamin D intake from foods.

In the U.S., milk is fortified with vitamin D3, and it has been suggested that adolescents can maintain adequate serum 25(OH)D by drinking the recommended amount of milk daily [16]. However, there are concerns regarding that suggestion since the recommendations for adolescents are three servings/day [11,17]. Since one 8-ounce glass of milk contains only about 100 IU of vitamin D3, drinking three glasses would provide only ~300 IU of vitamin D3. An adolescent would need to consume six glasses of fortified milk to meet the IOM recommendation of 600 IU of vitamin D per day. Further, many popular milks, such as soy and almond, are not consistently fortified with vitamin D. The reality is the intake of milk in the adolescent population is significantly less than what is recommended. In fact, most adolescent girls average less than one eight-ounce serving per day [18-20].

Studies have found a correlation between milk intake and serum 25(OH)D [5,6,21-22]. However, since the prevalent level of milk intake is low, it has been difficult to determine the potential impact of adequate milk intake to maintain sufficient levels of 25(OH)D. Our completed randomized trial of the effects of increasing milk and yogurt intake on changes in body weight in adolescent girls provided an opportunity to evaluate the relationship between milk intake and serum 25(OH)D levels in a cohort of adolescent girls.

Methods:

Study Design and Participants: This is a cross-sectional analysis of final visit data from a one-year NIH-funded randomized controlled trial. The purpose of that trial was to determine if increasing calcium intake to recommended levels with dairy foods in adolescent females with habitually low calcium intake would decrease body fat gain compared to similar females who continue their low calcium intake Lappe et al. (in press). Participants were 13 or 14 years old, at least one and one-half years post-menarcheal, had body mass index (BMI) percentile >50th and <98th, and reported eating two or less dairy servings per day. The Creighton University Institutional Review Board approved this project. Participant assent and parental consent were obtained.

Girls (n=274) were randomized into either the dairy or control group for one year of follow up. The dairy group was instructed to consume at least 1200 mg of calcium/day from low-fat milk and/or yogurt. The control group was asked to continue their current diet, which at baseline averaged less than 600 mg of calcium and less than one glass of milk per day. A registered dietician provided individual instruction on the dietary protocol to girls and a parent on enrollment and assessed intervention adherence at each of the four post-enrollment visits.

At the final visit (V5), serum 25(OH)D measurement was done on a subset of 124 girls (Dairy, N=61, and Control, N=63). The girls for this substudy were enrolled between January 2012 and September 2013. The racial breakdown of participants in this substudy was white 78% (N=97); black 14% (N=17); multi-racial 6% (N=7) and asian 2% (N=3).

Dietary Assessment: Assessment of dietary intake was done using the Nutrition Data System for Research (NDS-R) software, supported and updated by the Nutrition Coordinating Center, University of Minnesota. NDS-R features a dietary intake multiple-pass approach that prompts for complete food description, detailed food preparation, and diverse amount descriptions. Daily dietary recalls were obtained on two weekdays and one weekend day every three months. Intake was reviewed and confirmed by the dietician who also inquired about vitamin supplementation. None of the girls took calcium or vitamin D supplements. Individual daily intake from recalls collected at visits two through five were averaged.

25(OH)D: Serum 25(OH)D analyses were done at Creighton University Laboratory using the DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay. This is an immunoluminometric direct assay using directly coated magnetic micro particles to determine 25(OH)D levels. This laboratory is enrolled in the DEQAS quality assurance system for 25OHD assays and their findings on test samples are uniformly close to the international mean [23]. Blood was obtained by Registered Nurse through a venipuncture in the antecubital area.

Anthropometric Measurements: Height and weight were assessed at each visit. Height was assessed to the nearest 0.1 centimeter (cm) using a daily-calibrated, Harpenden stadiometer. Two measurements were obtained. If there was a difference between the two measurements greater than 1.0 cm, a third was taken, and the average of the two closest values was used. The reliability of the stadiometer at our center is 0.9995. Weight was assessed to the nearest 0.1 kilogram (kg) using an electronic scale (Scale-Tronix, Inc, White Plains NY) that was calibrated daily. Two measurements were obtained. If the difference between the two values was greater than 1.0 kg, a third measurement was taken and the average of the two closest values was used. BMI values were then calculated using the CDC BMI calculator based on age and gender [24].

Race: Race was self-reported by the participants and coded as either white or non-white. The categories were: Native American, Asian, Black, White, Multiracial and Other.

Season: Season of 25(OH)D measurement was determined by dividing the year into four seasons of three months each, based on that year's dates of the Northern Meteorological Seasons: Winter, December 1 to February 28; Spring, March 1 to May 31; Summer, June 1 to August 31; and Fall, September 1 to November 30. The girls were then placed in the category of which season their blood was drawn.

Statistical Analysis: Data analyses were performed using the Statistical Package for Social Sciences, version 22.0 (SPSS Inc. – Chicago, IL, United States). Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. Initially, t-tests were used to compare differences between the control and dairy groups, and multiple linear regression was used to investigate predictors of serum 25(OH)D. Covariates included average vitamin D intake, BMI, race, and season of blood draw. We tested for confounding and masking relationships by adding or removing variables and observing the effect on statistical significance of the remaining variables in the model.

Results: Seventy-two of the 124 girls (58%) had insufficient serum 25(OH)D levels below 30 ng/ml. Furthermore, 21 girls (17%) had 25(OH)D levels below 20 ng/ml and were considered deficient [10-12]. Over the course of the one-year study, girls in the Dairy group averaged 2.8 (SD ±1.03) servings of milk per day, while girls in the Control group averaged 0.67 (SD ±.498). Average milk intake among all sub study girls ranged from 0.0 to 4.5 servings per day. Dietary intake of vitamin D was 440 IU (SD ± 2.93) in the intervention group and 136 IU (SD ± 1.84) in the controls. There was a significant difference between the Dairy and the Control groups in dietary intake of vitamin D. But, there was not a statistically significant difference in serum 25(OH)D between the dairy group (29.59 ± 9.51 ng/mL) and the controls (27.95 ± 11.00 ng/mL; p = 0.38). Table 1 describes the means and standard deviations of the continuous variables used in the analysis for both the control and interventions groups as well as all the girls participating in the study.

	Control	**Intervention**	**All**
Serum 25(OH)D	27.95 (±11.0)	29.59 (±9.5)	28.76 (±10.29)
Milk Servings	.67/day (±.498)	2.8/day (±1.03)	1.7/day (±1.35)
Vitamin D Intake	136 IU/day (±72.0)	440 IU/day (±116.8)	284 IU/day (±180.4)
Body Mass Index	22.9 (±3.0)	23.2 (±3.0)	23.1 (±3.0)

Table 1: Descriptive Statistics (Means and Standard Deviations) for intervention group, control group, and all participants

The multiple linear regression analysis was conducted to examine the contribution of milk intake to serum 25(OH)D. Season, race, and BMI were entered as covariates. In the overall model, total variance explained by the model was 31% and milk intake did not predict serum 25(OH)D F (6, 117) β = .005; p = .95). However, when examining the covariates within the overall regression model, BMI, race (white vs not white), and season (summer) were all statistically significant predictors of serum 25(OH)D. The following at the betas and the significance for the covariates, respectively, (β = -0.21, p =.010; β = 0.32, p = .000; β = 0.39, p = .000) (Table 2). Although BMI was significant in the overall model, it did not moderate the nonsignificant association between milk intake and 25(OH)D levels. In other words, BMI is important when it comes to 25(OH)D but the nonsignificant association between milk intake and 25(OH)D was constant regardless of a girl's BMI.

Variables	B	SE B	Β	p-value
Season	8.8	2.2	.39	.000*
Race	7.8	2.1	.32	.000*
BMI	.71	.27	-.21	.010*
Milk Servings	.041	.633	.005	.959

*indicates statistical significance

Table 2: Summary of Simple Regression Analyses for Variables Predicting Serum 25(OH)D (N = 124)

These findings indicate that although drinking milk did not affect the serum 25(OH)D levels, the girls that have lower BMIs, are white, and had their blood drawn in the summer, were predicted to have significantly higher 25(OH)D levels.

Discussion: Our parent study gave us an opportunity to determine the effects of drinking milk on serum 25(OH)D in adolescent girls. Although the average milk intake of the dairy group over one year was nearly the recommended three servings per day and about twice that of the controls, there was no statistical or biological difference in serum 25(OH)D between the two groups. The findings from this study are consistent with NHANES reports which are also different from the IOM, AAP, and ES categories. These reports describe deficiency as less than 15 ng/ml and insufficiency as 15-29 ng/ml [5]. Within these categories, 58% of the girls in this study are considered vitamin D insufficient compared to 61% of NHANES, however this study demonstrates nearly double the amount of girls were deficient (17%) as compared to 9% in NHANES.

The mean vitamin D intake of the participants from all dietary sources was 284 IU per day which is 40% below the IOM recommendations of 600 IU per day. Overall, only 3% (N=4) of the girls met the 600 IU per day recommendation. This provides some understanding of why vitamin D insufficiency and deficiency are highly prevalent in adolescents and calls for more focus on vitamin D nutrition in adolescents and their parents. If those girls with insufficient levels are not identified and corrected, they could be at risk for health problems in adulthood.

Vitamin D experts suggest about 40 IU of vitamin D3 is needed to raise the serum 25(OH)D 3 ng/ml [25]. In other words, if an individual has a serum level of 20 ng/ml, that individual would need an additional 270 IU, on top of the recommended 600 IU/day, of vitamin D3/day to reach the desired level of 30 ng/ml. Further, overweight persons require more D3 to raise serum 25(OH)D an equivalent amount in normal weight persons [10]. Thus, it is not surprising that the girls in this study with higher milk intake did not increase their 25(OH)D by a significant amount since few of them were getting the recommended dietary vitamin D and at enrollment of the parent study, they all had a BMI above the 50% for weight.

Veugelers and Ekwaru propose that for individuals to achieve serum 25(OH)D levels of at least 20 ng/ml, they may need to be taking in approximately 7000 IU of vitamin D per day from all sources [26]. Unlike numerous other studies [6-8, 27], this study demonstrates most girls had sufficient levels of 25(OH)D (mean 28.8 ± 10.3 ng/mL) but less than half the girls (42%) had 25(OH)D levels above 30 ng/ml. This population of girls may still have additional nutritional needs if they are to receive health benefits associated with levels above 30 ng/ml. This further supports the concern that counseling adolescents to drink recommended servings of milk to main optimal serum 25(OH)D levels may not be sufficient.

Despite participation in a dairy study, many of these girls were still failing to meet the dairy intake recommendations. This is consistent with the trend in the literature of decreasing milk intake. On average participants were consuming less than 2 glasses of milk per day. Dror and Allen demonstrate that although there are significant health contributions with dairy consumption, there has been a steady decline and the trend is a decrease in intake with increasing age [18]. Therefore, with the current dietary intakes of adolescents, it may be difficult, if not impossible, for the healthy adolescent to obtain an adequate amount of vitamin D from dietary sources without supplementation and additional vitamin D fortification in dairy products. Very few studies have examined additional fortification of milk and serum vitamin D levels in children and adolescents but those that have, show improvements in 25(OH)D levels in those who consumed vitamin D fortified milk or food over various time periods [28,29].

The literature has demonstrated dark skinned and obese adolescents may require additional vitamin D at higher doses to obtain a sufficient serum 25(OH)D concentration [9]. Cashman notes that although nonwhite ethnic groups are at high risk for vitamin D deficiency, little research has been done in vitamin D status in minority adults and even less in children and adolescents [30]. In this study, the mean serum vitamin D for the African American girls was 17.7 (SD ± 8.8) ng/ml compared to the mean of the Caucasian girls which was 30.6 (SD ± 9.4). And when categorized as white or nonwhite, the means were 30.6 (SD ± 9.4) and 22.8 (SD ± 11.5) respectively.

Cediel et al also found that from pre-puberty to puberty onset, serum 25(OH)D levels decreased and those that had higher adiposity, also had lower 25(OH) levels [31]. This alludes to the importance of establishing adequate vitamin D levels prior to the onset of puberty and the potential adolescent adiposity increase. In another study of adolescents and obesity conducted by Shah, Wilson and Bachrach, 25(OH)D levels were associated with BMI and failed to increase even after high dose supplementation [32]. They conclude that future studies need to determine appropriate dosing for adolescents especially when overweight or obese.

Finally, individuals living in latitudes above or below 33 degrees with little to no sun exposure and conversion during many months of the year may also need extra supplementation or intervention [9]. In this study, the summer months were the only time of year that the mean serum levels were above 30 ng/ml again suggesting supplementation may be an important consideration during the remaining months of the year when girls may not be obtaining additional vitamin D from the other primary source of vitamin D, sunlight. Providers should consider discussing milk and vitamin D intake during all well-child visits but especially on an individual basis with those children who are overweight, those who are dark-skinned or those that have limited sun exposure and/or consistently use sunscreen. Vitamin D supplementation is an effective, low-cost, low-risk way of promoting health.

Limitations of this study are the cross-sectional design and lack of baseline serum 25(OH)D levels. Since the subjects were enrolled in a study in which they were asked to increase their milk and yogurt intake, they may not be representative of adolescent girls. The sample in general was a limited population of female adolescents living in a defined geographical area and who had a BMI between the 50th and 98th percentile. No data was collected within the parent study or the sub study regarding amount of sun exposure, sunscreen use or time spent outdoors. Finally, self- reported dietary intake can lead to possible recall errors.

Study strengths include the close monitoring of the participants to the protocol and the longitudinal nature of multiple, detailed dietary recalls validated by a registered dietician and analyzed using a highly recommended NDS-R system. Serum 25(OH)D was analyzed with the Liaison which has quality checks with DEQAS. Future studies should include baseline serum 25(OH)D, sunlight exposure and skin pigment assessments, and girls providing dietary recalls without a dietary intervention. Prospective studies should include these assessments as they contribute to serum 25(OH)D measures.

Conclusion: Milk is an important source for many key nutrients, including calcium and vitamin D. In this cohort, as in other studies, milk drinking was not associated with higher serum 25(OH)D levels in adolescent girls [9,18,29]. Although milk intake contributes to vitamin D status, this study suggests that even when girls were randomized to increase their dairy servings, they were still not getting enough to meet the current RDA for vitamin D. For adolescent girls to achieve adequate serum 25(OH)D levels and health benefits, providers need to evaluate individual serum 25(OH)D levels. For those with low levels, additional education and/or supplementation may be needed.

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