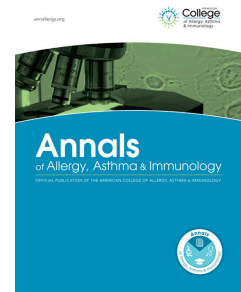


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Vitamin D and iron status in food allergic children

Sylwia Nowak, MD, Hongyue Wang, PhD, Brianne Schmidt, RD, Kirsi M. Jarvinen, MD, PhD



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Title:

Vitamin D and iron status in food allergic children

Author information:

Sylwia Nowak, MD¹

Hongyue Wang, PhD²

Brianne Schmidt, RD¹

Kirsi M. Jarvinen, MD, PhD¹

¹ Division of Pediatric Allergy and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York

² Department of Biostatistics and Computational Biology, University of Rochester Medical Center, Rochester, New York

Corresponding author:

Sylwia Nowak, MD

Division of Pediatric Allergy and Immunology

University of Rochester School of Medicine and Dentistry

Rochester, New York

Cell Phone (preferred): +1-773-603-0715

Work Phone: +1-312-563-2306

Fax: +1-312-563-2201 (attention: Sylwia Nowak)

Email: snowak925@gmail.com

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Abbreviations/Acronyms:

AR: allergic rhinitis

AD: atopic dermatitis

BMI: body mass index

CM: cow's milk

CMA: cow's milk allergy

EoE: eosinophilic esophagitis

FA: food allergy

FPIES: food protein-induced enterocolitis syndrome

Hgb: hemoglobin

IDA: iron deficiency anemia

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

TIBC: total iron binding capacity

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BACKGROUND : Children with food allergy are at specific risk for nutritional deficiencies.

OBJECTIVE : To retrospectively determine prevalence of vitamin D and iron deficiencies in children with or without food allergy (FA).

METHODS : We compared the markers of vitamin D and iron status of 0-17-year-olds with CMA (n=77), other FA (n=70), and atopic children without FA (n=87) at an academic pediatric allergy practice. Multiple linear regression analyses were performed to determine the impact of CMA and other FA on vitamin D levels and iron markers. **RESULTS :** Vitamin D deficiency was detected in one-fourth and insufficiency in onethird of children with CMA and other FA and in those with atopic diseases but no FA. Vitamin D levels were associated with vitamin D supplementation, and consumption of breast milk, cow's milk, infant formula or plant-based milk beverage, but not with CMA or other FA. Older children with FA who did not consume any cow's milk or alternative milk beverage were at highest risk for vitamin D insufficiency. CMA children have a higher rate of IDA (8%) than other FA children (1%) or those with no FA (5%, $p<0.001$); however suboptimal levels of transferrin saturation and iron were detected in up to one-third of children with CMA or other FA.

CONCLUSION : Vitamin D deficiency/insufficiency is common in atopic children overall but children with CMA are at higher risk for IDA. Intensive nutritional counseling and nutrient intake monitoring, specifically for vitamin D and iron in those avoiding cow's milk, is necessary to optimize nutritional status.

INTRODUCTION

Vitamin D is not only known for its effects on calcium and phosphorus metabolism,¹ bone development,² and relationship to lung development,³ but there is also a growing awareness of its potential role as an essential micronutrient with immunomodulatory effects.^{4,5} It is thought to improve antimicrobial defenses within the immune system, suppress excessive inflammation and inhibit production of pro-inflammatory cytokines.⁶ Various factors like diet, ethnicity, sun exposure, gender and obesity can affect vitamin D levels in children,^{3,4} and as many as 15% of children are found to have vitamin D insufficiency or deficiency based off of local laboratory cutoffs.⁷ However, the exact immunologic mechanisms driving the role of vitamin D in food allergy development has not yet been established.⁶

In the United States, prevalence of food allergies is anywhere from 4-8%,^{8,9} and worldwide, allergic diseases are on the rise.^{6,10} Although the data is conflicting, studies have shown that vitamin D levels, whether deficient or excessive, may play a role in the development of allergic diseases such as asthma, atopic dermatitis, and food allergy.¹¹ For instance, it has been suggested that vitamin D deficiency could be related to increased asthma risk and rising allergies.³ Multiple studies have also reported links between low vitamin D levels and increased sensitization to food allergens in infants and young children.^{12,13} One population-based cohort study out of Australia showed that infants with low vitamin D levels (<50 nmol/L at 1 year of age) had an increased risk of challenge-confirmed peanut and egg allergy at 1 year (11-fold peanut and 4-fold egg).¹² A cross-sectional study from Korea found that low vitamin D levels carried increased risk of food allergen sensitization and was associated with severity of atopic dermatitis. Interestingly,

severity of atopic dermatitis was influenced by vitamin D levels and by food allergy sensitization. On the other hand, a small case-control study in Australia did not find an association between vitamin D insufficiency at birth or 6 months of age and food allergy at 12 months of age.¹³

In contrast, vitamin D deficiency can occur in patients with already established food allergy, including cow's milk allergy due to decreased intake as a result of strict elimination of a notably good source of vitamin D. A study utilizing NHANES data reported an association between vitamin D deficiency and higher levels of total and allergen-specific IgE levels to aeroallergens and foods in children and adolescents.¹⁴ Similarly, a study from Brazil noted that children with cow's milk allergy, especially those that were breastfed, had a highest rate of vitamin D deficiency compared to healthy (non-allergic) controls,¹⁶ and a review study looking at elimination diets noted that children with multiple food allergies seem to be at greatest risk for inadequate intake of nutrients including vitamin D.¹⁵ Finally, children with non-IgE mediated gastrointestinal food allergy appear to be similarly at greater risk of vitamin D deficiency, as well.¹⁷

As eluded above, children with dietary restrictions are at risk for multiple nutrient deficiencies, not only vitamin D. In food allergic children, foods rich in essential nutrients for growth and development are often foods that contain the most common allergens.¹⁸ Those with milk allergies or multiple food allergies are at greatest risk with specific food avoidance measures possibly contributing to certain nutrient deficiencies. For instance, one review article cited that milk allergic children were found to have a lower intake of calcium, zinc and vitamin B2, whereas

children with allergy to wheat and soy also had lower iron, zinc and vitamin B6 levels.¹⁵ In comparison, children with beef, pork and chicken allergies had lower iron intake and iron levels noted.¹⁵ Lastly, a retrospective chart review study out of Taiwan urged consideration of milk protein allergy in children that presented for evaluation of iron deficiency anemia.¹⁹

Overall, current data is conflicting with regards to the role of vitamin D in food allergy. Consequently, the objective of this study was to determine whether vitamin D deficiency is more prevalent in children with food allergies when compared against patients with other atopic conditions but without food allergies (no FA). The food allergic group was further subdivided to separate children with cow's milk allergy (CMA) and those with food allergies other than cow's milk allergy (other FA). We also sought to assess other laboratory markers of nutritional status, such as iron status and factors associated with these nutritional markers.

METHODS

Study patients

We conducted a retrospective chart review where we examined 500 randomly selected charts of children that presented to the University of Rochester Medical Center Pediatric Allergy/Immunology clinic (Rochester, NY) aged 0 through 17 years with FA, asthma, allergic rhinitis (AR), atopic dermatitis (AD), food protein-induced enterocolitis syndrome (FPIES), and/or eosinophilic esophagitis (EoE) between January 2015 and December 2017. The study was approved by the Institutional Review Board of University of Rochester Medical Center. Children of both genders, all races and ethnicities were included. Children with FA were clinically

diagnosed by convincing history of a reaction and positive skin prick testing or serum specific IgE measurements. Children with non-IgE mediated conditions such as FPIES and EoE were included in the FA group, as well, as they were avoiding foods that can impact nutritional status. Children without FA who carried the diagnosis of other atopic conditions such as AR, AD, or asthma were used as the control group. Children with non-atopic co-morbidities that could affect vitamin D intake, absorption or utilization, those with chronic disease other than allergic disease, corticosteroid use in the past month, and children with FA that did not have nutritional laboratory tests including vitamin D levels drawn or had incomplete anthropometric data were excluded.

Data collected

Skin prick testing to foods including milk, egg, wheat, soy, peanut, tree nuts, fish, and shellfish with a wheal at least 3 mm larger than negative control were considered positive. Specific IgE antibody values of at least 0.35 IU/mL to environmental allergens were considered positive. Presence of multiple food allergy was noted in addition to number of foods eliminated (ranging from zero to eight corresponding to the most common allergens). Routine laboratory assessments performed within 6 months from the visit were used for determination of serum levels of serum 25-hydroxyvitamin D, iron, transferrin and its saturation, ferritin, total iron binding capacity (TIBC), hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and peripheral eosinophil count expressed as a percentage and absolute number. As defined by the Endocrine Society, serum 25-hydroxyvitamin D deficiency is set as less than or equal to 20 ng/mL with insufficiency defined as 21 to 29 ng/mL.²⁵ Although levels above 20 ng/mL are necessary to prevent rickets and osteomalacia, levels should be above 30 ng/mL in order to achieve maximal

vitamin effects for calcium levels and bone metabolism, especially in growing children.²⁵ In order to account for lab measurement assay variability and to guarantee sufficient vitamin D levels for optimal health, we used insufficiency of less than 30 ng/mL rather than deficiency in our study.²⁴ Per WHO, iron deficiency anemia (IDA) is hemoglobin (Hgb) <11g/dL in children < 5yo and ferritin <12mcg/L or Hgb <11.5g/dL and ferritin <15mcg/L in children 5-11yo.²⁶ As a cutoff for suboptimal iron stores, we used the following: iron <60 mcg/dL, transferrin saturation <20% or TIBC >240 mcg/dL.²⁷ Use of milk beverage (cow's milk, breast milk, plant-based alternative, formula, or none) was recorded from provider medical notes in the medical record. In addition, the name of consumed milk product was recorded, and ranged from plant-based milks to amino acid infant formulas depending on age and choice made by parent and provider. AD severity was based on the physical examination and assessment. Age, gender, race/ethnicity, body mass index percentile (BMI), and smoking status were obtained from medical charts and recorded as well.

Statistical analysis

All statistical analyses were conducted using SAS version 9.4. (SAS Institute Inc. Cary, NC). Subject demographics and characteristics were compared between children with CMA, other FA, and those with no FA. Fisher's exact test or the chi-square test was used for categorical variables while the one way ANOVA or Kruskal-Wallis tests were used for continuous variables. Post hoc pairwise comparisons were also performed using Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis. Multiple linear regression analyses with nutritional markers levels as dependent variables were performed to evaluate the association between laboratory markers and FA, age, gender, smoke exposure, milk beverage or alternative and vitamin D

supplementation. The type of milk beverage consumed was included in the analysis. All tests were two-sided at the 0.05 level of significance.

RESULTS

Study subject characteristics

Of the 500 charts reviewed, 234 children were included in the study. The remainder, had missing anthropometric or laboratory data. Among those, 147 children met criteria of CMA and other FA, and 87 children represented patients seen for other atopic conditions but no FA. Because this study occurred in a tertiary referral center for food allergies, the proportion of food allergic children was high in this random selection of patients. Out of all the children with FA, there were 55% children allergic to cow's milk, 23% to wheat, 47% to egg, 52% to peanuts, 45% to tree nuts, 27% to soy, 9.8% to fish, and 17% had an allergy to shellfish. Because presence of CMA by itself can carry significant nutritional repercussions in children, those in the food allergic group were separated into two groups: "CMA" and "Other FA". These groups included subjects with non-IgE-mediated FA. Notably, 34 children with EoE and 13 with FPIES had IgE-mediated sensitization on testing as well. There were 4 children with EoE and 2 with FPIES that did not have positive skin testing or serologic testing to foods; all were included in the other FA group.

Table 1 compares the baseline characteristics of the three groups studied. Children with FA were younger, compared to children with no FA ($p<0.001$). Children with CMA were significantly younger (5.0 years) than children with other FA (8.1 years) and children with no FA (8.5 years),

p=xxx. This is expected, as younger children are more likely to carry a CMA label. Gender was not found to be significantly different between the three groups. Our clinic carries a diverse patient population, so African American and Hispanic patients were well represented in our cohort, accounting for 23% of the children with CMA, 40% of children with other FA, and 46% of children with no FA. There was a higher rate of Caucasians in the CMA group and of Hispanics in no FA group ($p=0.005$). Children with CMA had the lowest BMI (median 52.5 %ile) compared to children with other FA (54.3 %ile) and no FA (63.5 %ile) ($p=0.015$). This difference in BMI is expected, as children with CMA were younger. Presence of smoke exposure was low in all groups, but appeared to be less prevalent in children with CMA (4%) and other FA (17%) than in children with no FA (22%, $p=0.004$).

Less children with any FA consumed CM (67% other FA group) and more CM alternatives (19% other FA and 87% CMA group) when compared to 77% children with no FA ($p<0.001$). The CMA group substituted CM with mostly plant-based alternatives (58%) and formula (21%, $p<0.001$). Thirteen to fourteen percent of children in each group did not consume any CM alternative. There was no statistically significant difference in vitamin D supplementation consumption in the three groups.

There was an overall high prevalence of other co-morbid allergic diagnoses in each group, which is often the case in patients seeking care at academic institutions. 64% of children with other FA and 74% of children with CMA had multiple IgE-mediated FA ($p<0.001$). The most commonly avoided additional foods in the CMA group included wheat (10%), egg (36%), peanut (49%), tree nuts (39%) and soy (31%), whereas the most commonly avoided foods in the other FA

group included egg (53%), peanut (51%), tree nuts (44%), and soy (14%). In fact, wheat allergy was reported in 22 children (31%) with other FA, and in 8 children (10%) with CMA ($p=0.006$). Egg allergy was reported in 37 (53%) children with other FA and 28 (36%) children with CMA ($p=0.200$). There was no statistically significant difference in the proportion of children diagnosed with AD or asthma between groups, but the rate of moderate AD was more common in children with other FA and severe AD was more common in children with CMA ($p=0.014$), whereas AR was most common in children with no FA ($p<0.001$). Children with CMA and other FA were found to have higher blood eosinophil counts ($p=0.002$) and eosinophil percentages ($p=0.002$) (Table 2), consistent with their degree of atopy.

Nutritional markers

Children with CMA and other FA had no significant difference in vitamin D levels compared to children with no FA (Table 2). More specifically, 25 (32%) children with CMA, 27 (39%) children with other FA, and 35 (37%) children with no FA were vitamin D insufficient. Vitamin D deficiency was noted in 18 (23%) children with CMA, 16 (23%) children with other FA, and 11 (13%) children with no FA, comparable between groups. Hgb and ferritin levels were comparable between the three groups. However, 6 (8%) children with CMA, 1 (1%) child with other FA, and 4 (5%) children with no FA met IDA criteria ($p<0.001$). Transferrin saturation and iron levels as markers of depleted iron stores were lowest in children with other FA (mean 22.7% and 66.6 mcg/dL, respectively) and highest in those with no FA (28.0% and 79.7 mcg/dL, respectively), ($p=0.004$ and $p=0.026$, respectively). Iron deficiency without associated anemia as evidenced by low transferrin saturation levels were found in 25 (32%) children with CMA, 26

(37%) children with other FA and 15 (17%) children with no FA ($p=0.013$), or as evidenced by low iron levels in 19 (25%) children with CMA, 20 (29%) children with other FA and 12 (14%) children with no FA ($p=0.063$). MCHC was also found to be marginally lower in children with other FA ($p=0.010$).

Multiple linear regression was then used to evaluate the association between vitamin D levels and the presence of CMA and other FA, and factors such as age, gender, smoke exposure, type of milk, and vitamin D supplement. The presence of CMA or other FA, age and smoke exposure did not significantly impact vitamin D levels (Table 3). However, as predicted, presence of a dietary or supplemental vitamin D source was associated with higher levels of vitamin D. Vitamin D oral supplementation appeared to have a significant positive influence on vitamin D levels (mean estimate 8.36, 95% CI [6.37-10.36], $p<0.001$, Table 3). Similarly, children that consumed CM or any form of CM alternative had higher vitamin D levels than those without any milk beverage (Figure 1). Breastfed infants were supplemented with vitamin D in 88% of the time and children that consumed breast milk had the highest vitamin D level estimates (mean estimate 7.33, 95% CI [1.06-13.59], $p=0.022$), with CM providing the next highest vitamin D level (mean estimate 5.41, 95% CI [2.25-8.57], $p<0.001$). As expected, formula made a bigger difference on vitamin D levels (mean estimate 4.78, 95% CI [0.69-8.86], $p=0.022$) compared to a plant-based milk alternative (mean estimate 3.99, 95% CI [0.18-7.81], $p=0.04$). The most common CM alternatives were found to be soy and almond milk. Interestingly, two-thirds (67%) of children with other FA that did not consume a milk alternative to CM were 10 years and older ($n=18$). Of those patients, 83% were vitamin D insufficient.

Multiple regression analysis was also performed to analyze the impact of FA and other factors on transferrin saturation levels as an indicator of depleted iron stores (Table 4). There was a significant negative association between presence of other FA and transferrin saturation status (mean difference -5.27, 95% CI [-8.46- -2.08]). CMA, age, gender, smoke exposure, types of milk beverage consumed versus none, and presence of vitamin D supplementation were not found to have significant effect on transferrin saturation levels.

DISCUSSION

Our study showed that although vitamin D insufficiency appeared to be common in atopic children and was seen in one-third of those evaluated in our office, vitamin D levels were actually comparable between children with CMA, other FA, and those with no FA. Elimination of CM protein did not seem to be detrimental to vitamin D levels likely due to frequent supplementation with CM alternatives (which are often vitamin D fortified) or vitamin D supplements (especially in breastfed infants), as diets which contained a milk alternative and/or a vitamin D supplement tend to yield higher serum vitamin D levels. Despite the different types of plant-based and formula-based substitutes reported, our data showed that any substitute exerted a positive benefit on vitamin D levels, although this effect was attenuated depending on the type of milk alternative consumed. We can only assume that the milk substitute choices were vitamin D fortified (as most are) but as there were no food diaries utilized, we could not say for certain if the specific brands chosen were fortified. This trends with the amount of fortification, nutritional additives, and perhaps even bioavailability in infant formulas compared to plant-based alternatives. We also showed that IDA was uncommon in these groups, although iron and

transferrin saturation levels as an indicator of decreasing iron stores were lower in children with other FA, compared to those with CMA or no FA.

Vitamin D insufficiency was particularly prevalent (83%) in older children (>10 years) with any FA, who were not on a CM alternative beverage. These children had been on elimination diets for longer and because CM alternative is often considered non-crucial in management of older children who otherwise have varied diets, may actually contribute to the high rate of deficiency in this group. Additionally, children with other FA were in this group; they may have “outgrown” their CMA, but may have not incorporated CM back into the diet out of preference. In fact, the DRACMA guidelines to date recommend calcium supplementation over age 2 and not necessarily vitamin D supplementation or CM alternative supplementation in those that avoid CM.²⁷ This finding may carry weight in the school-aged group as well as in potentially all other age groups that do not consume CM alternative, or take a vitamin supplement. This was also confirmed in one study looking at vitamin D levels in patients with EoE and found that those with insufficient vitamin D levels tended to be older, although that study included both adults and children.⁷ A simple question then during a visit with such children can quickly signal that screening blood work or immediate initiation of an acceptable milk alternative is necessary. Allergies to some of the other common foods like egg that are higher in vitamin D could further exacerbate deficiency, and if not adequately substituted,²⁵ could predispose to lower vitamin D levels. Our study also showed that children with FA appeared to have higher eosinophil count and percentage, as eosinophils are a driver of concurrent atopic conditions (AR, AD) and speak to the severity of allergic condition. However, eosinophil counts did not contribute to the vitamin D or transferrin saturation levels.

This study also showed that children with CMA have a higher rate of IDA (8%) than children with other FA (1%), although the rate of IDA in those with no FA was in between (5%). In addition, all FA patients had lower transferrin saturation and iron levels, most pronounced in the other FA group. In up to one-third of children with any FA those levels were suboptimal. This may be due to other FA that put children at risk for insufficient iron intake, such as wheat or egg allergy. In fact, more wheat allergic children were found in the other FA group. However, this was not clinically important, because the rate of IDA was lowest in children with other FA. Transferrin saturation is a marker that may indicate decreasing iron stores, with levels below 20% indicative of subclinical or increased risk for IDA; however, iron stores (assessed by normal ferritin) appeared to be intact in these FA children. This may suggest that iron levels should be cautiously monitored in this group, as presence of other FA like wheat allergy can restrict ingestion of iron-fortified grains and cereals. It has also been shown that children with predominantly GI associated CMA manifestations that may lead to occult blood loss are at risk for this deficiency.¹⁹ Transferrin saturation and ferritin levels can also be influenced by inflammatory conditions, so transferrin saturation levels should be used in the context of other markers for iron status. One study found that there was an attenuated association between iron deficiency and vitamin D deficiency in healthy children,²⁰ although our study suggests that these mechanisms may be different.

Consequently, a physician and dietician team approach may be beneficial for surveillance of nutritional status²¹ not only in infants and toddlers but in older children with FA in order to identify nutritional deficiencies like vitamin D deficiency and to provide patient-specific

recommendations of dietary changes such as identifying appropriate dairy-free alternatives.^{18, 22}

One study found that CM avoidance was associated with inadequate calcium intake and those that received counseling about appropriate substitutions with alternative formula or milk were less likely to develop inadequate calcium and vitamin D levels.²³ The same principle and approach can be applied to other nutritional parameters that can emerge in children with CMA or multiple FA to diet staples in a particular age range.

Some strengths of this study include that we had a well-represented cohort of both genders, ages, and ethnicities in both groups, in whom markers of nutritional status had been commonly documented. Adjusting for confounders allowed for a closer look at statistically significant differences and their impact on the nutritional markers. Due to a large percentage of children with CMA in our cohort, we were able to subdivide the FA group into a CMA and other FA group so that a more rigorous statistical analysis was able to be performed to assess the implications a CM elimination diet can have on nutritional status. A key limitation in this study was that it was retrospective in nature, relying on clinical notes available to determine severity of allergic co-morbidities. Dietary intake was not measured by food diaries which would provide a more precise evaluation on the type and amount of milk alternatives consumed (and their vitamin D content). Another limitation is that other variables that could affect vitamin D levels such as sun exposure, seasonal timing of when the testing was performed, and other micro- and macronutrient factors were not collected and analyzed. Interestingly, children with CMA were somewhat younger compared to children with no FA, but the age was adjusted for in the logistic regression analysis, with no influence on vitamin D or transferrin saturation levels. Larger studies across all pediatric ages will be needed to determine if these findings can be validated

across larger populations of children. More in-depth vitamin D studies focusing on older children with FA should be done to evaluate for risk factors for vitamin D deficiency and downstream effects. Furthermore, categorizing children by combinations of FA may offer more insight on nutritional status rather than by pure number of eliminated foods in these studies.

In conclusion, although vitamin D deficiency appeared to be common in atopic children, vitamin D levels were comparable in children with CMA, other FA and no FA. Importantly, vitamin D levels were higher in those patients who had vitamin D supplementation, drank CM, or found a CM alternative as a vitamin D source in comparison to those without an alternative milk beverage or oral supplement. Especially older children with CMA were at high risk for vitamin D insufficiency. Moreover, lower iron and transferrin saturation levels appeared to be associated with FA. This underscores the need for close monitoring by a physician/dietitian team for proper management of children with FA to ensure they meet their nutritional and vitamin needs as well as to determine standardized algorithms for vitamin D testing that go beyond clinician suspicion for nutritional deficiency.

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Table 1

General characteristics of entire study population (n=234)				
Characteristic	No FA, n=87	Other FA, n=70	CMA, n=77	P-value
Age (y), median (range)	8.5 (1.2-17)	8.1 (0.6-17)	5.0 (0.5-17)	<0.001
Gender				0.514
Male, n (%)	58 (67%)	41 (59%)	51 (66%)	
BMI (%ile), median (range)	63.5 (1-140)	54.3 (0.1-110)	52.5 (0.1-119)	0.015
Race/Ethnicity				0.005
African American, n (%)	24 (28%)	25 (36%)	11 (14%)	
Caucasian, n (%)	43 (49%)	36 (51%)	53 (69%)	
Hispanic, n (%)	16 (18%)	3 (4%)	7 (9%)	
Other, n (%)	4 (5%)	6 (9%)	6 (8%)	
Smoke Exposure				0.004
Yes, n (%)	19 (22%)	12 (17%)	3 (4%)	
Type of Milk Beverage				<0.001
Cow milk, n (%)	67 (77%)	47 (67%)	0 (0%)	
Cow milk alternative, n (%)	8 (9%)	13 (19%)	67 (87%)	
None, n (%)	12 (14%)	10 (14%)	10 (13%)	
Type of Milk Alternative				<0.001
Breastmilk, n (%)	0 (0%)	3 (4%)	5 (7%)	
Cow milk, n (%)	67 (77%)	47 (68%)	1 (1%)	
Formula, n (%)	4 (5%)	7 (10%)	16 (21%)	
Plant-based alternative, n (%)	4 (5%)	3 (4%)	45 (58%)	
None, n (%)	12 (13%)	10 (14%)	10 (13%)	
Vitamin D Supplementation				0.262
Yes, n (%)	38 (44%)	22 (31%)	32 (42%)	
Multiple Food Allergies				<0.001
Yes, n (%)	1 (1%)	45 (64%)	57 (74%)	
Atopic Dermatitis				0.209
Yes, n (%)	48 (55%)	46 (66%)	52 (68%)	
Atopic Dermatitis Severity				0.014
None, n (%)	39 (45%)	24 (34%)	25 (33%)	
Mild, n (%)	33 (38%)	23 (33%)	28 (36%)	
Moderate, n (%)	15 (17%)	19 (27%)	14 (18%)	
Severe, n (%)	0 (0%)	4 (6%)	10 (13%)	
Allergic Rhinitis				<0.001
Yes, n (%)	82 (94%)	51 (73%)	34 (44%)	
Asthma				0.143
Yes, n (%)	47 (54%)	35 (50%)	30 (39%)	

P-value from a chi-square test, Fisher's exact test, one-way ANOVA or Kruskal–Wallis test
 Abbreviation: BMI, body mass index; FA, food allergy; CMA, cow's milk allergy

Table 2

Comparison of laboratory data between children with CMA and other FA compared to children with no FA				
	No FA, mean (SD)	Other FA, mean (SD)	CMA, mean (SD)	P-value
Vitamin D levels	28.45 (7.60)	26.50 (8.98)	27.55 (9.52)	0.323
Iron	79.72 (31.60)	66.64 (23.72)	75.29 (27.69)	0.026
Ferritin	52.77 (40.25)	46.02 (38.83)	48.26 (31.59)	0.376
Transferrin saturation	28.02 (11.44)	22.70 (8.74)	23.73 (8.90)	0.004
TIBC	333.57 (70.06)	340.47 (61.91)	347.10 (45.97)	0.467
Hemoglobin	15.12 (17.19)	13.53 (9.95)	12.18 (1.23)	0.113
Hematocrit	37.38 (4.50)	37.46 (5.30)	36.17 (3.26)	0.077
MCH	27.80 (2.94)	27.43 (2.83)	27.75 (2.13)	0.617
MCHC	33.49 (1.21)	33.07 (1.33)	33.70 (1.16)	0.010
MCV	82.16 (6.78)	84.71 (33.15)	82.23 (5.57)	0.378
Eosinophil count	0.27 (0.31)	0.44 (0.46)	0.46 (0.47)	0.002
Eosinophil %	3.03 (2.64)	5.01 (4.54)	5.26 (4.75)	0.002

Shown is mean (standard deviation); Bold indicates p-value <0.05, one way ANOVA or Kruskal–Wallis tests were used.

Abbreviation: TIBC, total iron binding capacity; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; FA, food allergy; CMA, cow's milk allergy

Table 3

Multiple linear regression analysis evaluating effects of CMA and other FA on vitamin D levels				
Parameter	Mean Difference	95% CI		Pr > t
Food allergy (yes vs no)	-0.86	-3.20	1.49	0.472
Cow milk allergy (yes vs no)	-0.40	-3.73	2.94	0.814
Age (years)	-0.12	-0.35	0.10	0.282
Gender (F vs M)	-1.72	-3.68	0.25	0.086
Smoke exposure (yes vs no)	0.34	-2.39	3.08	0.804
Type of milk beverage (BM vs none)	7.33	1.06	13.59	0.022
Type of milk beverage (CM vs none)	5.41	2.25	8.57	<0.001
Type of milk beverage (Formula vs none)	4.78	0.69	8.86	0.022
Type of milk beverage (Plant based alternative vs none)	3.99	0.18	7.81	0.040
Vitamin D supplement (yes vs no)	8.36	6.37	10.36	<0.001

Abbreviation: CI, confidence interval; CM, cow's milk; FA, food allergy; CMA, cow's milk allergy; BM, breast milk

Table 4

Multiple linear regression analysis evaluating effects of CMA, other FA and transferrin saturation on vitamin D levels				
Parameter	Mean Difference	95 % CI		Pr > t
Food allergy (yes vs no)	-5.27	-8.46	-2.08	0.001
Cow milk allergy (yes vs no)	-4.37	-8.92	0.17	0.059
Age (years)	0.23	-0.07	0.53	0.138
Gender (F vs M)	1.45	-1.23	4.13	0.286
Smoke exposure (yes vs no)	-3.21	-6.93	0.52	0.091
Type of milk beverage (BM vs none)	-0.46	-9.00	8.08	0.915
Type of milk beverage (CM vs none)	2.98	-1.32	7.28	0.174
Type of milk beverage (Formula vs none)	3.88	-1.69	9.45	0.171
Type of milk beverage (Plant based alternative vs none)	3.69	-1.51	8.88	0.163
Vitamin D supplement (yes vs no)	0.80	-1.92	3.52	0.563

Abbreviation: CI, confidence interval; CM, cow's milk; FA, food allergy; CMA, cow's milk allergy

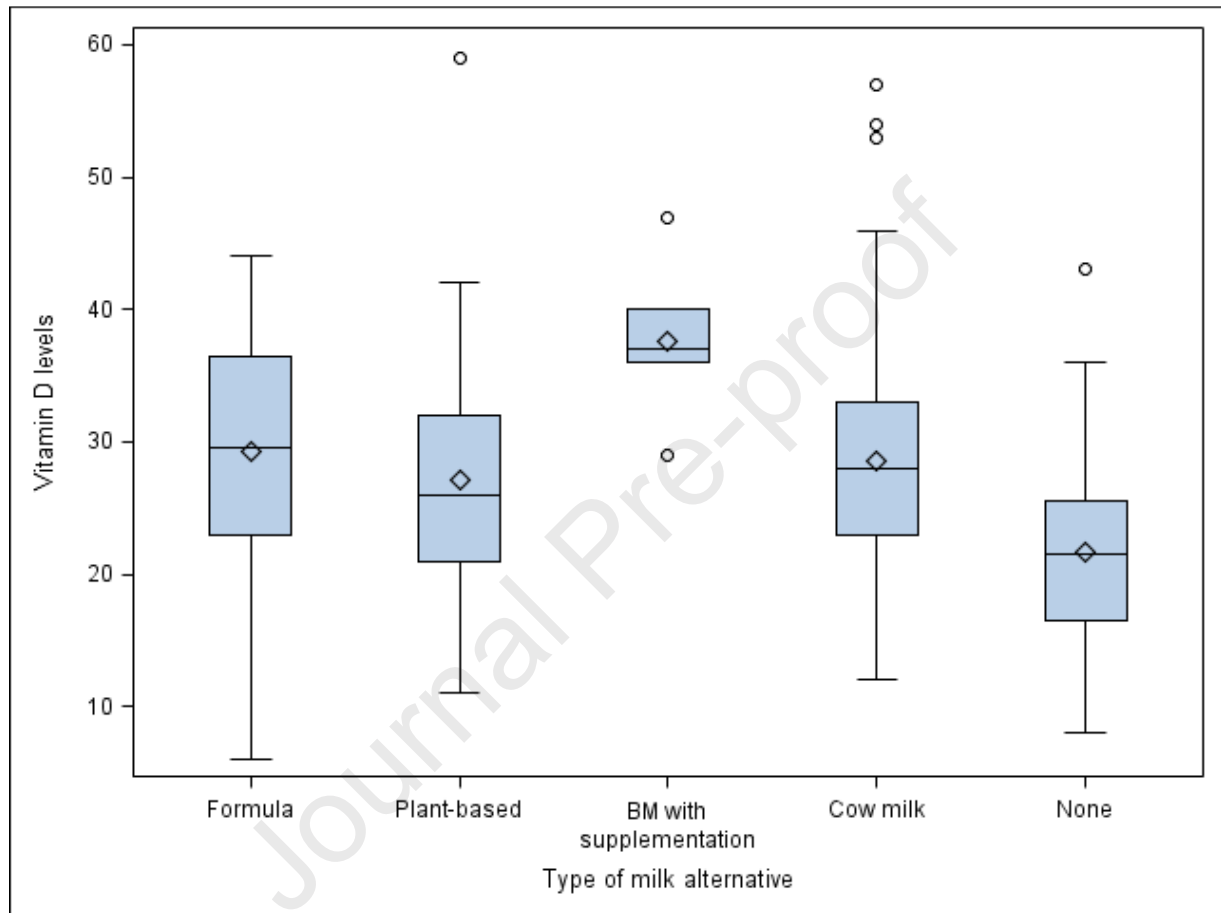
Figure 1

Figure 1

Title: Boxplots of Vitamin D levels of all cow's milk alternatives

Caption: Seven breastfed infants were also receiving vitamin D supplementation. Vitamin D levels: in ng/mL. Open diamond: mean vitamin D level. Vertical line inside box: median (50th percentiles). Vertical lines constituting top and bottom of box: 25th and 75th percentiles, respectively.

INTRODUCTION

Vitamin D is not only known for its effects on calcium and phosphorus metabolism,¹ bone development,² and relationship to lung development,³ but there is also a growing awareness of its potential role as an essential micronutrient with immunomodulatory effects.^{4,5} It is thought to improve antimicrobial defenses within the immune system, suppress excessive inflammation and inhibit production of pro-inflammatory cytokines.⁶ Various factors like diet, ethnicity, sun exposure, gender and obesity can affect vitamin D levels in children,^{3,4} and as many as 15% of children are found to have vitamin D insufficiency or deficiency based off of local laboratory cutoffs.⁷ However, the exact immunologic mechanisms driving the role of vitamin D in food allergy development has not yet been established.⁶

In the United States, prevalence of food allergies is anywhere from 4-8%,^{8,9} and worldwide, allergic diseases are on the rise.^{6,10} Although the data is conflicting, studies have shown that vitamin D levels, whether deficient or excessive, may play a role in the development of allergic diseases such as asthma, atopic dermatitis, and food allergy.¹¹ For instance, it has been suggested that vitamin D deficiency could be related to increased asthma risk and rising allergies.³ Multiple studies have also reported links between low vitamin D levels and increased sensitization to food allergens in infants and young children.^{12,13} One population-based cohort study out of Australia showed that infants with low vitamin D levels (<50 nmol/L at 1 year of age) had an increased risk of challenge-confirmed peanut and egg allergy at 1 year (11-fold peanut and 4-fold egg).¹² A cross-sectional study from Korea found that low vitamin D levels carried increased risk of food allergen sensitization and was associated with severity of atopic dermatitis. Interestingly,

severity of atopic dermatitis was influenced by vitamin D levels and by food allergy sensitization. On the other hand, a small case-control study in Australia did not find an association between vitamin D insufficiency at birth or 6 months of age and food allergy at 12 months of age.¹³

In contrast, vitamin D deficiency can occur in patients with already established food allergy, including cow's milk allergy due to decreased intake as a result of strict elimination of a notably good source of vitamin D. A study utilizing NHANES data reported an association between vitamin D deficiency and higher levels of total and allergen-specific IgE levels to aeroallergens and foods in children and adolescents.¹⁴ Similarly, a study from Brazil noted that children with cow's milk allergy, especially those that were breastfed, had a highest rate of vitamin D deficiency compared to healthy (non-allergic) controls,¹⁶ and a review study looking at elimination diets noted that children with multiple food allergies seem to be at greatest risk for inadequate intake of nutrients including vitamin D.¹⁵ Finally, children with non-IgE mediated gastrointestinal food allergy appear to be similarly at greater risk of vitamin D deficiency, as well.¹⁷

As eluded above, children with dietary restrictions are at risk for multiple nutrient deficiencies, not only vitamin D. In food allergic children, foods rich in essential nutrients for growth and development are often foods that contain the most common allergens.¹⁸ Those with milk allergies or multiple food allergies are at greatest risk with specific food avoidance measures possibly contributing to certain nutrient deficiencies. For instance, one review article cited that milk allergic children were found to have a lower intake of calcium, zinc and vitamin B2, whereas

children with allergy to wheat and soy also had lower iron, zinc and vitamin B6 levels.¹⁵ In comparison, children with beef, pork and chicken allergies had lower iron intake and iron levels noted.¹⁵ Lastly, a retrospective chart review study out of Taiwan urged consideration of milk protein allergy in children that presented for evaluation of iron deficiency anemia.¹⁹

Overall, current data is conflicting with regards to the role of vitamin D in food allergy. Consequently, the objective of this study was to determine whether vitamin D deficiency is more prevalent in children with food allergies when compared against patients with other atopic conditions but without food allergies (no FA). The food allergic group was further subdivided to separate children with cow's milk allergy (CMA) and those with food allergies other than cow's milk allergy (other FA). We also sought to assess other laboratory markers of nutritional status, such as iron status and factors associated with these nutritional markers.

METHODS

Study patients

We conducted a retrospective chart review where we examined 500 randomly selected charts of children that presented to the University of Rochester Medical Center Pediatric Allergy/Immunology clinic (Rochester, NY) aged 0 through 17 years with FA, asthma, allergic rhinitis (AR), atopic dermatitis (AD), food protein-induced enterocolitis syndrome (FPIES), and/or eosinophilic esophagitis (EoE) between January 2015 and December 2017. The study was approved by the Institutional Review Board of University of Rochester Medical Center. Children of both genders, all races and ethnicities were included. Children with FA were clinically

diagnosed by convincing history of a reaction and positive skin prick testing or serum specific IgE measurements. Children with non-IgE mediated conditions such as FPIES and EoE were included in the FA group, as well, as they were avoiding foods that can impact nutritional status. Children without FA who carried the diagnosis of other atopic conditions such as AR, AD, or asthma were used as the control group. Children with non-atopic co-morbidities that could affect vitamin D intake, absorption or utilization, those with chronic disease other than allergic disease, corticosteroid use in the past month, and children with FA that did not have nutritional laboratory tests including vitamin D levels drawn or had incomplete anthropometric data were excluded.

Data collected

Skin prick testing to foods including milk, egg, wheat, soy, peanut, tree nuts, fish, and shellfish with a wheal at least 3 mm larger than negative control were considered positive. Specific IgE antibody values of at least 0.35 IU/mL to environmental allergens were considered positive. Presence of multiple food allergy was noted in addition to number of foods eliminated (ranging from zero to eight corresponding to the most common allergens). Routine laboratory assessments performed within 6 months from the visit were used for determination of serum levels of serum 25-hydroxyvitamin D, iron, transferrin and its saturation, ferritin, total iron binding capacity (TIBC), hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and peripheral eosinophil count expressed as a percentage and absolute number. As defined by the Endocrine Society, serum 25-hydroxyvitamin D deficiency is set as less than or equal to 20 ng/mL with insufficiency defined as 21 to 29 ng/mL.²⁵ Although levels above 20 ng/mL are necessary to prevent rickets and osteomalacia, levels should be above 30 ng/mL in order to achieve maximal

vitamin effects for calcium levels and bone metabolism, especially in growing children.²⁵ In order to account for lab measurement assay variability and to guarantee sufficient vitamin D levels for optimal health, we used insufficiency of less than 30 ng/mL rather than deficiency in our study.²⁴ Per WHO, iron deficiency anemia (IDA) is hemoglobin (Hgb) <11g/dL in children < 5yo and ferritin <12mcg/L or Hgb <11.5g/dL and ferritin <15mcg/L in children 5-11yo.²⁶ As a cutoff for suboptimal iron stores, we used the following: iron <60 mcg/dL, transferrin saturation <20% or TIBC >240 mcg/dL.²⁷ Use of milk beverage (cow's milk, breast milk, plant-based alternative, formula, or none) was recorded from provider medical notes in the medical record. In addition, the name of consumed milk product was recorded, and ranged from plant-based milks to amino acid infant formulas depending on age and choice made by parent and provider. AD severity was based on the physical examination and assessment. Age, gender, race/ethnicity, body mass index percentile (BMI), and smoking status were obtained from medical charts and recorded as well.

Statistical analysis

All statistical analyses were conducted using SAS version 9.4. (SAS Institute Inc. Cary, NC). Subject demographics and characteristics were compared between children with CMA, other FA, and those with no FA. Fisher's exact test or the chi-square test was used for categorical variables while the one way ANOVA or Kruskal-Wallis tests were used for continuous variables. Post hoc pairwise comparisons were also performed using Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis. Multiple linear regression analyses with nutritional markers levels as dependent variables were performed to evaluate the association between laboratory markers and FA, age, gender, smoke exposure, milk beverage or alternative and vitamin D

supplementation. The type of milk beverage consumed was included in the analysis. All tests were two-sided at the 0.05 level of significance.

RESULTS

Study subject characteristics

Of the 500 charts reviewed, 234 children were included in the study. The remainder, had missing anthropometric or laboratory data. Among those, 147 children met criteria of CMA and other FA, and 87 children represented patients seen for other atopic conditions but no FA. Because this study occurred in a tertiary referral center for food allergies, the proportion of food allergic children was high in this random selection of patients. Out of all the children with FA, there were 55% children allergic to cow's milk, 23% to wheat, 47% to egg, 52% to peanuts, 45% to tree nuts, 27% to soy, 9.8% to fish, and 17% had an allergy to shellfish. Because presence of CMA by itself can carry significant nutritional repercussions in children, those in the food allergic group were separated into two groups: "CMA" and "Other FA". These groups included subjects with non-IgE-mediated FA. Notably, 34 children with EoE and 13 with FPIES had IgE-mediated sensitization on testing as well. There were 4 children with EoE and 2 with FPIES that did not have positive skin testing or serologic testing to foods; all were included in the other FA group.

Table 1 compares the baseline characteristics of the three groups studied. Children with FA were younger, compared to children with no FA ($p<0.001$). Children with CMA were significantly younger (5.0 years) than children with other FA (8.1 years) and children with no FA (8.5 years).

This is expected, as younger children are more likely to carry a CMA label. Gender was not found to be significantly different between the three groups. Our clinic carries a diverse patient population, so African American and Hispanic patients were well represented in our cohort, accounting for 23% of the children with CMA, 40% of children with other FA, and 46% of children with no FA. There was a higher rate of Caucasians in the CMA group and of Hispanics in no FA group ($p=0.005$). Children with CMA had the lowest BMI (median 52.5 %ile) compared to children with other FA (54.3 %ile) and no FA (63.5 %ile) ($p=0.015$). This difference in BMI is expected, as children with CMA were younger. Presence of smoke exposure was low in all groups, but appeared to be less prevalent in children with CMA (4%) and other FA (17%) than in children with no FA (22%, $p=0.004$).

Less children with any FA consumed CM (67% other FA group) and more CM alternatives (19% other FA and 87% CMA group) when compared to 77% children with no FA ($p<0.001$). The CMA group substituted CM with mostly plant-based alternatives (58%) and formula (21%, $p<0.001$). Thirteen to fourteen percent of children in each group did not consume any CM alternative. There was no statistically significant difference in vitamin D supplementation consumption in the three groups.

There was an overall high prevalence of other co-morbid allergic diagnoses in each group, which is often the case in patients seeking care at academic institutions. 64% of children with other FA and 74% of children with CMA had multiple IgE-mediated FA ($p<0.001$). The most commonly avoided additional foods in the CMA group included wheat (10%), egg (36%), peanut (49%), tree nuts (39%) and soy (31%), whereas the most commonly avoided foods in the other FA

group included egg (53%), peanut (51%), tree nuts (44%), and soy (14%). In fact, wheat allergy was reported in 22 children (31%) with other FA, and in 8 children (10%) with CMA ($p=0.006$). Egg allergy was reported in 37 (53%) children with other FA and 28 (36%) children with CMA ($p=0.200$). There was no statistically significant difference in children diagnosed with AD or asthma between groups, but the rate of moderate AD was more common in children with other FA and severe AD was more common in children with CMA ($p=0.014$), whereas AR was most common in children with no FA ($p<0.001$). Children with CMA and other FA were found to have higher blood eosinophil counts ($p=0.002$) and eosinophil percentages ($p=0.002$) (Table 2), consistent with their degree of atopy.

Nutritional markers

Children with CMA and other FA had no significant difference in vitamin D levels compared to children with no FA (Table 2). More specifically, 25 (32%) children with CMA, 27 (39%) children with other FA, and 35 (37%) children with no FA were vitamin D insufficient. Vitamin D deficiency was noted in 18 (23%) children with CMA, 16 (23%) children with other FA, and 11 (13%) children with no FA, comparable between groups. Hgb and ferritin levels were comparable between the three groups, as well. However, there were 6 (8%) children with CMA, 1 (1%) child with other FA, and 4 (5%) children with no FA that met IDA criteria ($p<0.001$). Transferrin saturation and iron levels as markers of depleted iron stores were lower in children with other FA (iron levels median 66 mcg/dL, transferrin saturation median 22%) compared to no FA (iron levels median 75 mcg/dL, transferrin saturation median 27%), with the lowest levels found in children with other FA ($p=0.004$ and $p=0.026$, respectively). Iron deficiency without

associated anemia as evidenced by low transferrin saturation levels were found in 25 (32%) children with CMA, 26 (37%) children with other FA and 15 (17%) children with no FA ($p=0.013$), or as evidenced by low iron levels in 19 (25%) children with CMA, 20 (29%) children with other FA and 12 (14%) children with no FA ($p=0.063$). MCHC was also found to be marginally lower in children with other FA ($p=0.010$).

Multiple linear regression was then used to evaluate the association between vitamin D levels and the presence of CMA and other FA, and factors such as age, gender, smoke exposure, type of milk, and vitamin D supplement. The presence of CMA or other FA, age and smoke exposure did not significantly impact vitamin D levels (Table 3). However, as predicted, presence of a dietary or supplemental vitamin D source was associated with higher levels of vitamin D. Vitamin D oral supplementation appeared to have a significant positive influence on vitamin D levels (mean estimate 8.36, 95% CI [6.37-10.36], $p<0.001$, Table 3). Similarly, children that consumed CM or any form of CM alternative had higher vitamin D levels than those without any milk beverage (Figure 1). Breastfed infants were supplemented with vitamin D in 88% of the time. More precisely, children that consumed breast milk had the highest vitamin D level estimates (mean estimate 7.33, 95% CI [1.06-13.59], $p=0.022$), with CM providing the next highest vitamin D level (mean estimate 5.41, 95% CI [2.25-8.57], $p<0.001$). As expected, formula made a bigger difference on vitamin D levels (mean estimate 4.78, 95% CI [0.69-8.86], $p=0.022$) compared to a plant-based milk alternative (mean estimate 3.99, 95% CI [0.18-7.81], $p=0.04$). The most common CM alternatives were found to be soy and almond milk. Interestingly, two-thirds (67%) of children with other FA that did not consume a milk alternative to CM were 10 years and older ($n=18$). Of those patients, 83% were vitamin D insufficient.

Multiple regression analysis was also performed to analyze the impact of FA and other factors on transferrin saturation levels as an indicator of depleted iron stores (Table 4). There was a significant negative association between presence of other FA other than CMA and transferrin saturation status (mean difference -5.27, 95% CI [-8.46- -2.08]). CMA, age, gender, smoke exposure, types of milk beverage consumed versus none, and presence of vitamin D supplementation were not found to have significant effect on transferrin saturation levels.

DISCUSSION

Our study showed that although vitamin D insufficiency appeared to be common in atopic children and was seen in one-third of those evaluated in our office, vitamin D levels were actually comparable between children with CMA, other FA, and those with no FA. Elimination of CM protein did not seem to be detrimental to vitamin D levels likely due to frequent supplementation with CM alternatives (which are often vitamin D fortified) or vitamin D supplements (especially in breastfed infants), as diets which contained a milk alternative and/or a vitamin D supplement tend to yield higher serum vitamin D levels. Despite the different types of plant-based and formula-based substitutes reported, our data showed that any substitute exerted a positive benefit on vitamin D levels, although this effect was attenuated depending on the type of milk alternative consumed. We can only assume that the milk substitute choices were vitamin D fortified (as most are) but as there were no food diaries utilized, we could not say for certain if the specific brands chosen were fortified. This trends with the amount of fortification, nutritional additives, and perhaps even bioavailability in infant formulas compared to plant-based

alternatives. We also showed that IDA was uncommon in these groups, although iron and transferrin saturation levels as an indicator of decreasing iron stores were lower in children with other FA, compared to those with CMA or no FA.

Vitamin D insufficiency was particularly prevalent (83%) in older children (>10 years) with any FA, who were not on a CM alternative beverage. These children had been on elimination diets for longer and because CM alternative is often considered non-crucial in management of older children who otherwise have varied diets, may actually contribute to the high rate of deficiency in this group. Additionally, children with other FA were in this group; they may have “outgrown” their CMA, but may have not incorporated CM back into the diet out of preference. In fact, the DRACMA guidelines to date recommend calcium supplementation over age 2 and not necessarily vitamin D supplementation or CM alternative supplementation in those that avoid CM.²⁷ This finding may carry weight in the school-aged group as well as in potentially all other age groups that do not consume CM alternative, or take a vitamin supplement. This was also confirmed in one study looking at vitamin D levels in patients with EoE and found that those with insufficient vitamin D levels tended to be older, although that study included both adults and children.⁷ A simple question then during a visit with such children can quickly signal that screening blood work or immediate initiation of an acceptable milk alternative is necessary. Allergies to some of the other common foods like egg that are higher in vitamin D could further exacerbate deficiency, and if not adequately substituted,²⁵ could predispose to lower vitamin D levels. Our study also showed that children with FA appeared to have higher eosinophil count and percentage, as eosinophils are a driver of concurrent atopic conditions (AR, AD) and speak

to the severity of allergic condition. However, eosinophil counts did not contribute to the vitamin D or transferrin saturation levels.

This study also showed that children with CMA have a higher rate of IDA (8%) than children with other FA (1%), although the rate of IDA in those with no FA was in between (5%). In addition, all FA patients had lower transferrin saturation and iron levels, most pronounced in the other FA group. In up to one-third of children with any FA those levels were suboptimal. This may be due to other FA that put children at risk for insufficient iron intake, such as wheat or egg allergy. In fact, more wheat allergic children were found in the other FA group. However, this was not clinically important, because the rate of IDA was lowest in children with other FA.

Transferrin saturation is a marker that may indicate decreasing iron stores, with levels below 20% indicative of subclinical or increased risk for IDA; however, iron stores (assessed by normal ferritin) appeared to be intact in these FA children. This may suggest that iron levels should be cautiously monitored in this group, as presence of other FA like wheat allergy can restrict ingestion of iron-fortified grains and cereals. It has also been shown that children with predominantly GI associated CMA manifestations that may lead to occult blood loss are at risk for this deficiency.¹⁹ Transferrin saturation and ferritin levels can also be influenced by inflammatory conditions, so transferrin saturation levels should be used in the context of other markers for iron status. One study found that there was an attenuated association between iron deficiency and vitamin D deficiency in healthy children,²⁰ although our study suggests that these mechanisms may be different.

Consequently, a physician and dietician team approach may be beneficial for surveillance of nutritional status²¹ not only in infants and toddlers but in older children with FA in order to identify nutritional deficiencies like vitamin D deficiency and to provide patient-specific recommendations of dietary changes such as identifying appropriate dairy-free alternatives.^{18, 22} One study found that CM avoidance was associated with inadequate calcium intake and those that received counseling about appropriate substitutions with alternative formula or milk were less likely to develop inadequate calcium and vitamin D levels.²³ The same principle and approach can be applied to other nutritional parameters that can emerge in children with CMA or multiple FA to diet staples in a particular age range.

Some strengths of this study include that we had a well-represented cohort of both genders, ages, and ethnicities in both groups, in whom markers of nutritional status had been commonly documented. Adjusting for confounders allowed for a closer look at statistically significant differences and their impact on the nutritional markers. Due to a large percentage of children with CMA in our cohort, we were able to subdivide the FA group into a CMA and other FA group so that a more rigorous statistical analysis was able to be performed to assess the implications a CM elimination diet can have on nutritional status. A key limitation in this study was that it was retrospective in nature, relying on clinical notes available to determine severity of allergic co-morbidities. Dietary intake was not measured by food diaries which would provide a more precise evaluation on the type and amount of milk alternatives consumed (and their vitamin D content). Another limitation is that other variables that could affect vitamin D levels such as sun exposure, seasonal timing of when the testing was performed, and other micro- and macronutrient factors were not collected and analyzed. Interestingly, children with CMA were

somewhat younger compared to children with no FA, but the age was adjusted for in the logistic regression analysis, with no influence on vitamin D or transferrin saturation levels. Larger studies across all pediatric ages will be needed to determine if these findings can be validated across larger populations of children. More in-depth vitamin D studies focusing on older children with FA should be done to evaluate for risk factors for vitamin D deficiency and downstream effects. Furthermore, categorizing children by combinations of FA may offer more insight on nutritional status rather than by pure number of eliminated foods in these studies.

In conclusion, although vitamin D deficiency appeared to be common in atopic children, vitamin D levels were comparable in children with CMA, other FA and no FA. Importantly, vitamin D levels were higher in those patients who had vitamin D supplementation, drank CM, or found a CM alternative as a vitamin D source in comparison to those without an alternative milk beverage or oral supplement. Especially older children with CMA were at high risk for vitamin D insufficiency. Moreover, lower iron and transferrin saturation levels appeared to be associated with FA. This underscores the need for close monitoring by a physician/dietitian team for proper management of children with FA to ensure they meet their nutritional and vitamin needs as well as to determine standardized algorithms for vitamin D testing that go beyond clinician suspicion for nutritional deficiency.

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Title:

Vitamin D and iron status in food allergic children

Author information:

Sylwia Nowak, MD¹

Hongyue Wang, PhD²

Brianne Schmidt, RD¹

Kirsi M. Jarvinen, MD, PhD¹

¹ Division of Pediatric Allergy and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York

² Department of Biostatistics and Computational Biology, University of Rochester Medical Center, Rochester, New York

Corresponding author:

Sylwia Nowak, MD

Division of Pediatric Allergy and Immunology

University of Rochester School of Medicine and Dentistry

Rochester, New York

Cell Phone (preferred): +1-773-603-0715

Work Phone: +1-312-563-2306

Fax: +1-312-563-2201 (attention: Sylwia Nowak)

Email: snowak925@gmail.com

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Abbreviations/Acronyms:

AR: allergic rhinitis

AD: atopic dermatitis

BMI: body mass index

CM: cow's milk

CMA: cow's milk allergy

EoE: eosinophilic esophagitis

FA: food allergy

FPIES: food protein-induced enterocolitis syndrome

Hgb: hemoglobin

IDA: iron deficiency anemia

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

TIBC: total iron binding capacity

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