Cow's Milk Allergy and Bone Mineral Density in Prepubertal Children

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abstract

BACKGROUND AND OBJECTIVES: Recent data suggest that cow's milk allergy (CMA) has become more persistent, prolonging treatment via strict elimination of cow's milk products into a period of skeletal growth. The objectives of this study were to compare bone mineral density (BMD), vitamin D status, and dietary intakes of calcium and vitamin D between prepubertal children with persistent CMA and those with non–cow's milk food allergies (NCMA) as control subjects and to assess the use of and compliance to calcium and vitamin D supplementation among children with persistent CMA.

METHODS: Fifty-two children with persistent CMA and 29 with NCMA were recruited. BMD was measured by using dual energy radiograph absorptiometry, and vitamin D status was assessed by using plasma 25-hydroxyvitamin D concentrations. Calcium and vitamin D intakes, as well as compliance to calcium and vitamin D supplementation, were recorded.

RESULTS: Lumbar spine BMD *z* scores were significantly lower in children with CMA. Low bone mass was detected in 6% of the CMA group compared with none in the NCMA group. Children with CMA displayed significantly lower calcium intakes than control subjects. Vitamin D status was not reduced in children with CMA compared with control subjects. Fewer than one-half of children with CMA reported the use of calcium and vitamin D supplements. However, adherence was high among supplement users, with a mean compliance rate of 5.5 days per week.

CONCLUSIONS: These prepubertal children with persistent CMA had lower lumbar spine BMD *z* scores than children with NCMA, which likely resulted from lower calcium intake.

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WHAT'S KNOWN ON THIS SUBJECT: Reduced bone density has been reported in adults with persistent cow's milk allergy (CMA). Bone density, vitamin D status, and intake and compliance to calcium and vitamin D supplementation have never been documented in prepubertal children with persistent CMA.

WHAT THIS STUDY ADDS: Prepubertal children with persistent CMA have a lower bone mineral density and calcium intake, but similar vitamin D status and intake, compared with similarly aged children with food allergies other than cow's milk.

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Cow's milk allergy (CMA) represents the most common food allergy during childhood, affecting 2% to 3% of children.[1,2] A strict elimination diet removing cow's milk, dairy products, and their traces remains the main treatment plan until allergy resolution. Although resolution of CMA in 87% of children by 3 years of age has been reported,[3] more recent evidence suggests persistence of allergy until adolescence in 15% of cases.[4,5] The deleterious impact of CMA on bone mineral density (BMD) has been documented in small cohorts of children and adolescents,[6,7] probably resulting from deprivation of key bone nutrients during a period of intensive skeletal growth.

Dairy products account for >50% of calcium and vitamin D intakes in children.[8] The elimination diet thus increases the risk of nutrient inadequacy[9] and renders calcium and vitamin D supplementation necessary in cases in which alternatives do not meet dietary requirements.[10] During growth, dietary calcium is required to maintain a positive calcium balance, essential for optimizing bone mass accretion. Many studies have shown a positive association between calcium intakes and bone mass acquisition in children.[11–14] It has been established that bone mass accretion during the years of rapid skeletal growth is a major determinant of adult bone health[15,16] and that failure to achieve peak bone mass is associated with increased risk of osteoporosis and fracture later in life.[17]

Vitamin D maximizes intestinal absorption of calcium, thus contributing to the maintenance of adequate mineral homeostasis and bone mineralization. Because the main source of vitamin D is from sunlight, limited exposure, particularly in winter and in higher latitudes, increases the dependence on dietary sources to provide adequate intakes. This situation is prevalent in Canada,[18] making vitamin D fortification mandatory for milk, and led us to question the vitamin D status among Quebec children with persistent CMA. This group is characterized by limited sun exposure during winter and potential dietary deficiency. Currently, there are no clinical guidelines requiring assessment of vitamin D status and BMD in children with CMA. The limited access to a dietitian in the Quebec public health system, as well as the inability of many parents to afford a consultation and follow-up by a dietitian in private clinics, complicates the situation.

Given that children with food allergies face important dietary restrictions, we conducted a cross-sectional study to determine whether prepubertal children with persistent CMA display suboptimal vitamin D status and impaired bone health compared with a group consisting of children with nondairy food allergies. In addition, the compliance to calcium and vitamin D supplementation was evaluated among participants, given that these factors are poorly documented. The results presented here provide a basis for the reassessment of the current management of food allergies.

METHODS

Study Design and Participants

The ethics committee of the Sainte-Justine University Hospital Center approved the protocol, and parents or legal guardians provided written consent.

Fifty-two prepubertal children with immunoglobulin E–mediated CMA and 29 prepubertal children with non–cow's milk food allergy (NCMA) were recruited at the allergy clinic of Sainte-Justine University Hospital Center between 2011 and 2014. To minimize the influence of sun exposure on 25-hydroxyvitamin D (25[OH]D) levels, the study was conducted during the winter months (October–April). CMA was defined according to a history of type I hypersensitivity reaction to milk, persistent positive skin prick test response to milk, and specific immunoglobulin E levels ≥0.35 kU/L (UniCAP; Pharmacia, Uppsala, Sweden) or ≥0.1 kU/L (Immulite; Siemens Healthcare Diagnostics, Munich, Germany). Children with a history of bone, endocrine, liver, or kidney diseases or who were taking medications that may interfere with vitamin D metabolism were excluded. Children who consumed milk or dairy products, even in small amounts, were also excluded. Puberty was assessed by using self-reported Tanner staging, and the onset of puberty was an exclusion criterion. Parents answered a questionnaire regarding the child's allergic history, fractures, breastfeeding, and current and past corticosteroid use. Height was assessed with a stadiometer and weight with an electronic scale. Raw values for height were transformed into age- and gender-matched *z* scores according to reference data.[19] Blood samples were collected at recruitment, and plasma was stored at −20°C until analysis.

Bone Mineral Assessment

Lumbar spine (LS) L_2–L_4 areal BMD and total body composition (lean mass and body fat) were measured by using dual-energy radiograph absorptiometry (DXA) with the Lunar Prodigy scanner (GE Lunar Corp, Madison, WI). Results were transformed into age- and gender-specific *z* scores by using the LUNAR Prodigy normative database and interpreted according to the guidelines of the International Society for Clinical Densitometry for pediatrics.[20] To adjust for bone size, volumetric bone mineral density (vBMD) was calculated by using Kröger's cylindrical model.[21] Age- and gender-specific reference data from a cohort of white European

TABLE 1 Study Population Characteristics

Characteristic	CMA (n = 52)	NCMA (n = 29)	P
Gender			.46
Male	64	55	
Female	37	45	
Age, y	6.9 ± 1.9	6.8 ± 1.5	.42
Ethnicity			.83
White	92	90	
African American	4	3	
Asian	4	7	
No. of food allergies			.51
Simple	14	21	
Multiple (≥2)	86	79	
Breastfeeding history	95[a]	75	.01
Exclusive breastfeeding,[b] mo	4.6 ± 1.9	4.7 ± 2.1	.87
Total breastfeeding, mo	12.4 ± 10.0	9.6 ± 7.3	.28
Other atopic manifestations			
Asthma	76	67	.44
Eczema	24	33	.18
Lifetime use of corticosteroids	86	96	.21
Topical	76	92	.12
Nasal	37	46	.46
Inhaled	71	71	.96
Oral	41	25	.20
Use of corticosteroids in the last year	86	96	.21
Topical	37	46	.46
Nasal	29	29	.96
Inhaled	57	63	.67
Oral	17	9	.37
Physical activity level, min/d			.40
<30	4	11	
30–60	25	19	
>60	71	70	

Data are presented as percentage of children or mean ± SD. Student's t tests and χ^2 tests were applied to compare children with CMA versus children with NCMA.

[a] Significantly different from the NCMA group.

[b] Without the use of infant formula.

children were used to calculate the vBMD z scores.[22]

Calcium and Vitamin D Intakes

Dietary calcium and vitamin D intakes were assessed by using a validated quantitative food frequency questionnaire (FFQ) administered by a single dietitian.[23,24] Vitamin and mineral supplement use, along with physical activity, were also included in the questionnaire. Analysis of the FFQ was based on values from the Canadian Nutrient File version 2010 and the US Department of Agriculture National Nutrient Database for Standard Reference.[25,26] The categorization of nutritional intake was conducted according to the latest dietary reference intakes for calcium and vitamin D.[18] Because a nutrient intake <67% of the recommended dietary allowance (RDA) should be improved,[27] intakes for calcium and vitamin D were compared with their respective RDA for age and considered inadequate if they failed to meet 67% of this value. Among participants taking calcium and vitamin D supplementation, compliance was evaluated by establishing the frequency of supplementation over a 1-week period. Participants were considered compliant when supplementation was observed ≥4 days per week.[28]

Vitamin D Status

Plasma 25(OH)D measurement was performed by using liquid chromatography–tandem mass spectrometry at the Sainte-Justine Clinical Biochemistry Laboratory. Vitamin D status was deemed optimal at 25(OH)D levels >75 nmol/L (30 ng/mL), and vitamin D deficiency was defined as levels <30 nmol/L (12 ng/mL) according to the Clinical Practice Guidelines of the Endocrine Society.[29]

Statistical Analysis

Sample size calculation was based on the expected difference in BMD between children with CMA and those with NCMA. This difference was calculated by subtracting the BMD of similarly aged milk-avoider children[30] from the reference value of prepubertal children of the same age.[22] A sample of at least 27 children per group was required to detect a group difference with a power of 80% and a type I error of 5%. Data normality was assessed by using the Shapiro-Wilk test. Categorical and normally distributed continuous variables are reported as percentages and means ± SDs, respectively. Differences between groups were evaluated by using Student's t test or the χ^2 test. Pearson's correlation coefficients were computed to examine associations between bone mineral status, vitamin D status, body composition, and dietary intakes. Significance level was set at $P < .05$.

RESULTS

Characteristics of the study population are shown in Table 1. Children with CMA and those with NCMA were similar with respect to gender, ethnicity distribution, age, and physical activity level. The use of steroids, especially inhaled preparations, was high among the participants but did not differ between groups. All children were at Tanner stage 1. Although breastfeeding duration did not differ significantly between groups, a higher proportion of children with CMA were breastfed than children with NCMA.

Anthropometric and bone densitometry measurements are

outlined in Table 2. Height, lean mass, body fat percentage, weight, and height for age were comparable between groups. LS BMD *z* scores were significantly reduced in children with CMA compared with control subjects. Low BMD (*z* scores less than or equal to −2 SDs) was detected in 6% of the CMA group compared with none in the NCMA group. All children with low BMD were white, and none experienced any fracture. Three CMA children reported a single fracture (ie, wrist, leg, clavicle) compared with 2 in the NCMA group (ie, elbow, humerus). All fractures occurred while participating in sports. BMD did not differ between children who experienced a fracture and those who did not.

Dietary intake for calcium and vitamin D are shown in Table 3. Calcium intake was significantly reduced in the CMA group, with >60% of children failing to meet the RDA for calcium for this age group (1000 mg/d). In fact, >20% of CMA children consumed less than two-thirds of this RDA. Vitamin D intakes in children with CMA and NCMA were well below the RDA for this vitamin (600 IU/d), with only 11.5% and 7.4% meeting the RDA, respectively. Only 37% and 44% of children with CMA reported taking a supplement containing either calcium or vitamin D. Among the children with CMA taking those supplements, compliance was respected in 83% of cases with an average compliance rate of 5.5 days per week. Calcium and vitamin D intakes were not correlated to any of the bone parameters in CMA children. The exception was an unexpected negative correlation between vitamin D intakes and LS BMD ($r = -0.284$; $P = .04$). LS BMD ($r = 0.526$; $P = .005$) and vBMD ($r = 0.444$; $P = .02$) were each positively correlated with calcium intake in the NCMA group.

Nine of the 52 children with CMA and 4 of the 29 children with NCMA had missing 25(OH)D data.

TABLE 2 Anthropometric Measurements and LS Bone Mineral Status in Children With and Without CMA

Characteristic	CMA ($n = 52$)	NCMA ($n = 29$)	*P*
Height, cm	118.7 ± 10.8	117.9 ± 11.9	.76
Weight, kg	22.6 ± 6.4	22.3 ± 5.7	.83
Relative height (*z* score)	−0.4 ± 0.9	−0.7 ± 1.3	.24
Body fat, %	14.6 ± 5.5	17.2 ± 6.8	.08
Lean mass, kg	17.7 ± 3.8	17.4 ± 3.7	.83
BMD (*z* score)	−0.718 ± 0.7[a]	−0.208 ± 1.0	.03
vBMD (*z* score)	−0.358 ± 1.0[a]	0.295 ± 1.4	.03
Bone status, %			.18
Normal (≥2 SD)	94	100	
Low bone mass (less than or equal to −2 SD)	6	0	

Data are presented as percentage of children or mean ± SD. Student's *t* tests and χ^2 tests were applied to compare children with CMA versus children with NCMA. BMC, bone mineral content.
[a] Significantly different from the NCMA group.

TABLE 3 Calcium and Vitamin D Intakes of Children With and Without CMA

Characteristic	CMA ($n = 52$)	NCMA ($n = 29$)	*P*
Calcium, mg	930.1 ± 368.4[a]	1434.7 ± 741.8	.002
Vitamin D, IU	299.4 ± 182.7	346.1 ± 164.6	.27
Calcium			
Met RDA	39[a]	74	.003
<67% RDA	21[a]	0	.01
Vitamin D			
Met RDA	12	7	.56
<67% RDA	73	63	.35
Use of calcium supplement	37	22	.19
Use of vitamin D supplement	44	33	.35
Supplementation compliance at ≥4 d/wk			
Yes	83	—	—
No	17	—	—
Compliance rate			
No. of days per week	5.5	—	—

Data are presented as percentage of children or mean ± SD. Student's *t* tests and χ^2 tests were applied to compare children with CMA versus children with NCMA.
[a] Significantly different from the NCMA group.

To assess potential bias arising from missing data, outcomes from children with and without missing values were compared. We found no significant differences or trends for any of the tested variables between children with missing 25(OH) D data and those with complete data sets. Interestingly, vitamin D status was similar in children with CMA compared with the control subjects (Table 4). However, >51% of the children with CMA displayed suboptimal vitamin D levels (<75 nmol/L [<30 ng/mL]), and 3 children had 25(OH)D levels corresponding to vitamin D deficiency (<30 nmol/L [<12 ng/mL]). This proportion of vitamin D insufficiency was similar in children with NCMA (44%; $P = .28$); however, none of the children were vitamin D deficient. Bone densities were similar between children with optimal and suboptimal vitamin D status.

DISCUSSION

Our study found that these children with CMA had lower LS BMD *z* scores than children with NCMA despite seemingly normal growth and nutritional status. Our observations are in line with other studies, which examined BMD in patients with CMA.[6,31–33] Jensen et al[6] found reduced BMD in Danish children and teenagers with

CMA for >4 years compared with reference values from a similarly aged healthy group. However, their sample spanned a wide age range (ie, 8–17 years of age) and included participants at different pubertal stages. The investigators attributed their findings to the reduced height-for-age in their study population. Such an explanation is unlikely in the current study, as we found no difference in height-for-age *z* scores between the 2 groups. Furthermore, we minimized the confounding effect of bone size by calculating the vBMD, a parameter not reported in the study by Jensen et al.[6] The lower values observed in children with CMA thus reflect reduced bone mineral per volume unit, which might indicate decreased bone accretion. A greater proportion of children with CMA than with NCMA had been breastfed, which may have had an impact on bone mass. However, this factor cannot explain our findings for 3 reasons. First, both exclusive and total breastfeeding duration did not differ between groups. Second, previous studies have shown that breastfeeding has instead a positive[34] or neutral[35–37] effect on prepubertal bone mass. Finally, comparison of BMD between breastfed and formula-fed children for each group revealed no differences (data not shown).

In addition to genetic factors, calcium intake is another important determinant of the vertebral BMD in prepubertal children.[38] In a randomized controlled trial performed in identical twins, Johnston et al[11] found that daily administration of calcium 1000 mg to 1 member of the pair resulted in a significant gain in bone mass after 3 years. This positive impact disappeared during and after puberty. As expected, children with CMA had lower calcium intake than control subjects, with 62% having intake below the RDA compared with 26% of the children with NCMA. Despite the strict elimination diet, only 37% reported taking a calcium supplement. Surprisingly, we found no evidence of an association between calcium intakes and bone measurements in the CMA group, whereas these variables were positively correlated in the NCMA group. As opposed to the CMA group, children with NCMA likely obtain their calcium from dairy sources, which also contain large amounts of key bone nutrients such as protein, magnesium, phosphorus, potassium, and zinc.[39] Consequently, the positive association found between BMD and calcium intakes in this group may reflect higher consumption of these nutrients. One might speculate that lifelong elimination of milk and dairy products results in a diet of lower quality, which adversely affects the BMD of children with CMA. Height, weight, and body composition were similar in the CMA and NCMA groups, however, which indicates that nutrients were supplied in sufficient amounts to ensure normal growth. Unfortunately, we were unable to assess the intakes of other nutrients because the FFQ used in this study was designed and validated to assess habitual intakes of calcium and vitamin D. Nevertheless, correlations should be interpreted carefully given the small sample size of the 2 groups.

Vitamin D intakes were low in both groups, with 89% and 93% of CMA and NCMA children, respectively, unable to meet the recommended intake. Although the prevalence of vitamin D insufficiency exceeded 50% among our participants, most of the children displayed a vitamin D status compatible with optimal bone health (>50 nmol/L [>20 ng/mL]), with only 3 children with CMA in the deficiency range. The mean 25(OH)D levels were only slightly below the cutoff point for vitamin D sufficiency, suggesting considerable variability in serum 25(OH)D levels. In fact, we observed a seasonal decline in 25(OH)D levels, with mean ± SD levels of 77 ± 20.9 nmol/L in the fall, 69 ± 19.3 nmol/L in the winter, and 68 ± 16.0 nmol/L in the spring, with similar sampling across seasons ($P = .54$). A Cochrane review showed that it is unlikely that vitamin D supplements are beneficial in children and adolescents with normal vitamin D levels.[40] However, at 25(OH)D levels <50 nmol/l, a greater calcium intake seems to be required to increase BMD.[41] In these growing children with CMA, nonoptimal calcium intake may potentially have a deleterious impact on bone mass accretion. Our interpretation remains limited by the fact that bone densitometric measurements reflect lifetime nutritional influences, whereas the reported intakes represented only a cross-sectional assessment of current intakes.

To our knowledge, this study is the first to document the prevalence of calcium and vitamin D supplement use among children with CMA. Despite calcium and vitamin D intakes below the RDA, fewer than one-half of the children with CMA reported consumption of supplemental calcium or vitamin D. However, compliance among the users was high, with supplements being consumed on average 5.5 days a week. Factors that may explain the

TABLE 4 Vitamin D Status in Children With and Without CMA

Status	CMA ($n = 43$)	NCMA ($n = 25$)	*P*
25(OH)D, nmol/L[a]	70.1 ± 21	75.1 ± 16.2	.31
Vitamin D status, %			.28
Optimal	42	56	
Suboptimal	51	44	
Deficiency	7	0	

Data are presented as percentage of children or mean ± SD. Student's *t* tests and χ^2 tests were applied to compare children with CMA versus children with NCMA. No statistical differences were found.

[a] To convert nmol/L to ng/mL, divide by 2.496.

low prevalence of supplement use among children with CMA were not specifically investigated in this study but may be related to inadequate and insufficient nutrition education. Our findings thus stress the importance of nutritional counseling and monitoring of children with CMA, as we anecdotally observed that 77% of a subset of children (n = 13) who received nutritional counseling were compliant to supplementation (data not shown).

The short-term consequences of a decreased BMD in prepubertal children with CMA are unclear. However, the crucial importance of childhood and adolescence for the achievement of peak bone mass, combined with the growing persistence of CMA and the accompanying suboptimal bone nutrient intakes, imply that such findings may have serious long-term implications for the bone health of these children. We cannot exclude the possibility that such differences in BMD may widen as children with CMA grow older, and these differences could result in suboptimal peak bone mass attainment in early adulthood. The vBMD z scores of children with CMA lagged behind control subjects by a difference of 0.65 SD. This finding is similar to the approximate 0.5 SD gap reported by Nachshon et al,[31] who compared LS BMD z scores of young adults with persistent CMA versus those of a control group. Moreover, they found that 27% of adults with CMA had osteoporosis, suggesting worsening of bone health as the children grew older.

Another explanation for the reduced BMD in these children with CMA could come from steroid use. The proportion of steroid-exposed children was high in both groups, with topical and inhaled steroids being more frequently used than oral steroids. Steroids (particularly oral steroids) decrease calcium absorption, hamper bone formation, and increase resorption, which may limit bone mass accrual.[42] However, when we adjusted for steroid use, the difference in BMD remained significant regardless of the therapy (data not shown). It is unlikely that these 2 groups differed in terms of cumulative steroid exposure because they displayed atopic manifestations in similar proportions.

In contrast to other studies, which have focused on milk avoiders[30] or included both prepubertal and pubertal children,[6,32,33] we included a well-defined population of prepubertal children with diagnosed CMA; with this approach, we removed the confounding effect of puberty on BMD. Furthermore, we studied atopic control subjects with similar characteristics, allowing us to control for the effects of age, physical activity, weight, lean mass, and steroid use on bone health. The fact that these 2 groups differed primarily in their intakes of calcium reinforces the idea that dietary factors might exert a nonnegligible impact on BMD in prepubertal children with CMA. Another limitation involves the use of Dutch reference data for the vBMD z scores. Bone densities of European children may differ from those of North American children. However, because they are the only data available, these reference statistics are recommended by the International Society for Clinical Densitometry.[20] Although we detected no differences in physical activity levels between the groups, this factor may also account for the differences observed in BMD.

CONCLUSIONS

The prepubertal children with persistent CMA in this study displayed lower BMD z scores despite normal growth, a finding that may arise from suboptimal calcium intake. Only follow-up studies will confirm a lower bone acquisition rate in these children.

ABBREVIATIONS

25(OH)D: 25-hydroxyvitamin D
BMD: bone mineral density
CMA: cow's milk allergy
DXA: dual-energy radiograph absorptiometry
FFQ: food frequency questionnaire
LS: lumbar spine
NCMA: non–cow's milk food allergy
RDA: recommended dietary allowance
vBMD: volumetric bone mineral density

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