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Plasma 25(OH)D concentration in children with autism spectrum disorder

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Abstract

Children with autism spectrum disorder (ASD) are reported to have decreased bone cortical thickness (BCT). Vitamin D plays an important physiological role in bone growth and development, so deficiency of vitamin D could contribute to decreased BCT. The goal of this study was to compare plasma 25(OH)D concentration in three groups of Caucasian males age 4 to 8 years old: (1) ASD and an unrestricted diet ($n=40$), (2) ASD and a casein-free diet ($n=9$), and (3) unaffected controls ($n=40$). No significant group differences were observed ($p=0.4$). However, a total of 54 (61%) of the children in the entire cohort had a plasma 25(OH)D concentration of less than 20ng/mL, similar to findings of low 25(OH)D concentrations in population-based studies. Children with ASD should be monitored for vitamin D deficiency.

Autism spectrum disorders (ASD) are a class of neurodevelopmental disorders characterized by impairments in communication and social reciprocity, and by the presence of restricted and repetitive interests and behaviors.¹ The prevalence of ASD is currently estimated to be 6.7 per 1000 school-age children in the United States,² making ASD and co-occurring conditions an important public health issue.

One condition that may occur more often in children with ASD than in children with typical development is vitamin D deficiency. Vitamin D is a fat-soluble vitamin, long known to be important in calcium homeostasis and bone health. As evidence has accumulated that vitamin D receptors are present in a wide variety of tissues, vitamin D deficiency has been implicated in numerous disease states.³ Having ASD could potentially put a child at greater risk of vitamin D deficiency secondary to dietary restrictions or decreased exposure to sunlight. Children with ASD may limit their own diet because of sensory aversions or restricted interests. The diet may also be restricted by parents to eliminate exposure to certain dietary proteins, such as the milk protein casein, in an attempt to treat the ASD symptoms.⁴ Children with ASD may have decreased exposure to sunlight because their

after-school hours are often devoted to table-based therapies, they do not commonly participate in organized outdoor sports, and their preferred leisure activities often involve video game, computer, or TV screens in an indoor setting.

We previously reported that children with ASD have decreased mean metacarpal bone cortical thickness (BCT) compared with a reference population.⁵ Based on this, our overarching hypothesis is that children with ASD are at risk of decreased bone mineral density compared with typically developing children. For this study our focus was limited to one component contributing to bone mineral density, vitamin D. The aim was to test the hypothesis that children with ASD have a lower concentration of circulating vitamin D – plasma 25(OH)D. We compared plasma concentration of 25(OH)D in Caucasian males with and without ASD and 25(OH)D concentration in males with ASD with and without a casein-free diet.

Methods

Participant enrollment has been described elsewhere.⁵ In brief, 75 Caucasian males (participants) aged 4 to 8 years with a clinical diagnosis of ASD confirmed by the Autism Diagnostic Observation Schedule⁶ were frequency-matched on age to typically developing males (controls) having intravenous (IV) catheters placed for outpatient tonsillectomies. Nine participants were identified as being on a casein-free diet at the time of study enrollment. All participants were living at home with parents.

At the study visit, height and weight were measured. A body mass index (BMI) z-score was calculated based on the Centers for Disease Control and Prevention 2000 growth charts for the United States.⁷ A 20ml blood sample was drawn into a tube containing EDTA, centrifuged and stored at -70°C .

Plasma specimens were available for 71 participants and 69 controls. The nine participants on a casein-free diet were identified and plasma on all nine of these children was assayed. A subset ($n=80$) of the remaining participants and controls was chosen by a computer-generated table of random digits. Sampling was carried out such that there were eight participants and eight controls per age group (4, 5, 6, 7, and 8y) giving a total of 40 participants and 40 controls. A priori power calculations indicated that this sample size would provide 80% power to detect a 10% difference in 25(OH)D concentration, using a two-tailed t-test, while controlling type I error rate to 5%. The study was approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center and parents provided informed consent for all participants.

Plasma 25(OH)D concentration was measured by radioimmunoassay using commercially available kits (DiaSorin, Inc, Stillwater, MN). The intra- and inter-assay coefficients of variation were 13 to 16%.

Analysis

Demographic and season of enrollment variables are reported as means (standard deviation) or proportions as appropriate. The distributions of the plasma 25(OH)D concentrations were not normal, so comparisons were made on $\log_{10}$ -transformed values. Participants with and without a casein-free diet were compared with controls in an analysis of variance (ANOVA). Potential covariates and confounders, including age, BMI z-score, use of vitamin supplements or antiepileptic medication, and season of enrollment were also examined to determine their effect on group differences.

Measured 25(OH)D values with calculated median and quartiles for each group were plotted for visual examination. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

The three groups, participants on a casein-free diet, participants not on a casein-free diet, and controls did not differ significantly by age, BMI z-score, or season of enrollment (Table I). Of the nine children on a casein-free diet, five were being given daily supplements; two contained calcium. Eight participants not on casein-free diets were also being given daily vitamins, three of which contained calcium. None of the controls were reported to be receiving vitamin supplements. Two participants not on casein-free diets were being treated with antiepileptic medication. None of the participants on the casein-free diet and none of the controls were taking antiepileptic medication.

The median 25(OH)D concentration for participants on a casein-free diet was 19.9ng/mL (range 10.8–31.0ng/mL); for participants not on a casein-free diet it was 19.6ng/mL (range 8.9–30.7ng/mL); for controls it was 17ng/mL (range 7.8–28.3ng/mL; Fig. 1).

Comparison of log₁₀-transformed values of 25(OH)D concentration by ANOVA did not reveal any significant group differences ($p=0.4$). On examination of contrasts, there were no significant differences between participants not on a casein-free diet and either controls ($p=0.2$) or participants on a casein-free diet ($p=0.6$). No group differences were observed when children receiving calcium-containing supplements ($n=5$) were removed ($p=0.4$) or those receiving any supplements ($n=13$) were removed ($p=0.4$).

The median 25(OH)D concentration did not differ significantly by season ($p=0.2$), and adjusting for season of study visit did not alter the results.

Discussion

In this cohort of 89 Caucasian males aged 4 to 8 years old, no differences were observed in the concentrations of 25(OH)D between participants with ASD and controls, and no effect of a casein-free diet was observed within the participant group (Fig. 1). Fifty-four children in the entire cohort (61%) had concentrations of less than 20ng/mL. This is the minimum concentration recommended by the American Academy of Pediatrics to ensure good bone health.⁸ For pre-pubertal children this is a critical deficiency that can have sequelae throughout the rest of their lives. The peri-pubertal period is a time of rapid bone development and remodeling when an individual acquires peak bone mass.⁹

Vitamin D deficiency has become a major health concern in our current society where sunblock and indoor activities have limited sun exposure for children, and dietary sources cannot make up the difference. Results of this study provide additional evidence to support that concern. We expected 25(OH)D concentrations to vary by season of enrollment since exposure to sunlight is so important for vitamin D synthesis, but this expected difference by season was not observed. This could simply be a function of sample size, since the study was not designed to detect this difference. However, this finding could also represent effective use of sunblock during warmer months, keeping the measured 25(OH)D values lower.

Children with ASD in this study were no more likely than their matched controls to have a low 25(OH)D concentration. These results must be interpreted in light of the limitations of the control group. The control children were likely to have had some degree of inflammation which could affect 25(OH)D concentration. They were, however, carefully screened and

excluded for steroid use or sleep apnea that might contribute to alterations in growth patterns.

The majority of children in this cohort, participants and controls, had low concentrations of 25(OH)D. This finding should be of concern to all providers of medical care to children, but especially to those managing the care of children with ASD. These children may be at risk of additional threats to calcium homeostasis and bone health as they age. Opportunities for active weight-bearing exercise in sunlight can be limited for children with ASD. At least a quarter of children with ASD will also have chronic gastrointestinal symptoms¹⁰ that could also have an impact on calcium absorption. In addition, up to 46% of them are likely to be diagnosed with co-occurring epilepsy.¹¹ Treatment with certain antiepileptic drugs, including valproic acid, is associated with reduced bone mineral density.¹²

As children with ASD age into adulthood they will be confronted by general chronic health issues such as osteoporosis that affect any aging population. However, these additional medical problems will present greater challenges in people with ASD. We need to better understand the risks and potential preventive measures that can be taken for those individuals while they are still children.

What this paper adds

- Children with and without ASD have low plasma concentrations of 25(OH)D
- 25(OH)D concentrations do not differ between children with ASD and typically developing controls

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Fig. 1.
Plasma concentration of 25(OH)D in Caucasian males with autism spectrum disorder with and without casein-free diet compared with typically developing matched controls.

Table I

Males with autism spectrum disorder with and without a casein-free diet compared with typically developing controls

	Participants on casein-free diet (<i>n</i> =9)	Participants on unrestricted diet (<i>n</i> =40)	Controls (<i>n</i> =40)	<i>p</i> value
Age mean (SD), y	5.4 (1.0)	6.5 (1.5)	6.6 (1.4)	0.06
Body mass index z-score, mean (SEM)	0.47 (0.2)	0.79 (0.2)	0.56 (0.2)	0.6
Season of enrollment, <i>n</i> (%)				
Summer, Jun–Aug	3 (33)	12 (30)	8 (20)	0.5
Autumn, Sep–Nov	2 (22)	5 (16)	7 (18)	0.7
Winter, Dec–Feb	2 (22)	5 (13)	14 (35)	0.1
Spring, Mar–May	2 (22)	18 (45)	11 (28)	0.2

SEM, standard error of the mean.