

Vitamin D Deficiency Is Prevalent in Girls and Women With Rett Syndrome

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ABSTRACT

Objectives: The aim of the study was to determine the prevalence of vitamin D deficiency and identify the relation between 25-hydroxyvitamin D (25-(OH)D) levels and the consumption of dietary sources of vitamin D or exposure to anticonvulsants in girls and women with Rett syndrome (RTT).

Subjects and Methods: Retrospective review of the medical records of 284 girls and women with RTT to determine serum 25-(OH)D and parathyroid hormone levels, nutritional status, dietary sources of vitamin D, exposure to anticonvulsants, degree of mobility, and *MECP2* status.

Results: Twenty percent of girls and women who were tested ($n = 157$) had 25-(OH)D levels < 50 nmol/L. Multivitamin supplements, vitamin D-fortified milk, and commercial formulas were consumed by 40%, 52%, and 54%, respectively. Anticonvulsants were used by 57%, and 39% ambulated independently. Median 25-(OH)D levels were lower in individuals who did not receive multivitamin supplements ($P < 0.05$) or commercial formulas ($P < 0.001$) than in those who did. Median 25-(OH)D levels differed ($P < 0.01$) among racial and ethnic groups, but the number in some groups was small. Nutritional status, use of anticonvulsants, degree of mobility, and *MECP2* status did not influence 25-(OH)D levels.

Conclusions: Vitamin D deficiency is prevalent in girls and women with RTT. The use of multivitamin supplements or commercial formulas is associated with improved vitamin D levels. Attention to vitamin D may enhance bone mineral deposition and reduce the frequency of bone fractures in these individuals.

Key Words: anticonvulsants, bone fracture, low bone mineral density, *MECP2* mutation, milk products, multivitamin supplements

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that occurs in 1.09 of every 10,000 girls and women (1). Mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*) can be identified in 90% to 95% of cases (2,3). The hallmark features of RTT are apparently normal development until 6 to 18 months of life, followed by a period of rapid developmental regression, loss of acquired speech, the replacement of purposeful hand use with hand stereotypies, and deceleration in head growth (4,5). Other features that may affect the clinical course of these individuals include growth abnormalities, seizures, autistic features, gait apraxia, and breathing abnormalities when awake.

As part of the growth abnormalities, girls and women with RTT have an increased risk of low bone mineral density, measured by dual-energy x-ray absorptiometry (6,7). The deficits in bone mineralization increase with advancing age (6). Deficits in lean body mass, the use of anticonvulsants, and poor ambulatory status may contribute to their bone mineral deficits (6,8). As a consequence, girls and women with RTT are at increased risk for skeletal fractures with advancing age (6). We estimate that 30% of these individuals have fractures by the age of 30 years based on national survey data (K.J. Motil, unpublished data).

Vitamin D is a prohormone essential for the absorption of calcium from the gut. Vitamin D deficiency has well-known adverse effects on bone health, including rickets in children and osteomalacia in adults (9,10). Vitamin D insufficiency in adults has been defined on the basis of parathyroid hormone (PTH) levels, calcium absorption, and bone mineral density (11–13). In adults, serum 25-hydroxyvitamin D (25-(OH)D) levels ≥ 100 nmol/L also have been associated with maximal suppression of PTH and reduced fracture rates (14). In contrast, the true prevalence of vitamin D deficiency in children is unknown, in part, because of the absence of functional biomarkers such as PTH in this age group (15). Limited data in adolescents suggest that total calcium absorption in adolescents correlates with serum 25-(OH)D levels in early but not pre- or late puberty (16). Fractional calcium absorption adjusted for calcium intake is higher at lower vitamin D intakes when serum values are < 50 nmol/L, presumably reflecting a compensatory response to increased PTH and 1,25-(OH)D levels (16). Preliminary data from our studies suggest that 25-(OH)D may be associated with bone density in girls and women with RTT (6).

The 2011 Report on Dietary Reference Intakes from the Institute of Medicine states that the recommended dietary allowance for vitamin D, 600 IU/day for ages 1 to 70 years corresponds to a serum 25-(OH)D level of at least 50 nmol/L and meets the requirement of at least 97.5% of the population based on available scientific evidence relating vitamin D to bone health during conditions of minimal sun exposure (17). In the context of the Institute of Medicine report, we designed the present study to determine the prevalence of vitamin D deficiency in girls and women with RTT

and identify factors that favorably or adversely influence the vitamin D status of these individuals. We hypothesized that vitamin D deficiency is prevalent in girls and women with RTT and low 25-(OH)D levels are associated with decreased consumption of dietary sources of vitamin D and increased exposure to anti-convulsant medications. We anticipate that attention to vitamin D nutrition in affected girls and women may reduce the risk of bone fractures in these individuals.

SUBJECTS AND METHODS

Subjects

Girls and women who met the clinical diagnostic criteria for RTT with or without a *MECP2* mutation, ages 1 to 60 years, were eligible for study (4,5). All of the individuals were followed through the Blue Bird Circle Rett Center at Texas Children's Hospital, Houston, and/or the Rare Diseases Clinical Research Network (<http://www.clinicaltrials.gov> identifier no. NCT00296312) at 1 of the 4 locations throughout the United States. Individuals whose medical or research records contained information about their dietary sources of vitamin D intake, the present use of anticonvulsant medications, and their degree of mobility were selected for study. The RTT cohort (n=284) was a sample of convenience based on the number of individuals studied by the investigators for clinical and research purposes between November 1, 2007 and December 31, 2009. Complete datasets for 25-(OH)D and PTH concentrations were available for a variable number of subjects because of accessibility to individual medical or research records and the information provided by primary care physicians.

The study was approved and a waiver of informed consent was granted by the institutional review board for human subject research at Baylor College of Medicine.

Study Design

The study design was observational. The medical and research records of a cohort of girls and women with RTT were reviewed retrospectively to determine the prevalence of low serum 25-(OH)D levels and identify risk factors associated with altered vitamin D status.

Procedures

All of the nutritional and clinical assessments were documented in the medical or research records using a structured questionnaire. Three investigators (K.J.M., S.P.G., and F.A.) were responsible for the nutritional assessments and measurements; the remaining investigators were responsible for the clinical assessments. The following data were obtained from the medical or research records: demographics, dietary sources of vitamin D, the present use of anticonvulsant medications, the degree of mobility, and laboratory values for 25-(OH)D and PTH.

Demographic Data

Demographic data included the individual's age, race or ethnicity, *MECP2* mutation, body mass index (BMI) z score as a measure of nutritional status, and location of the clinical or research site. Race and ethnicity were categorized as white (non-Hispanic), African American, Hispanic, Asian, and Native American. *MECP2* mutations were grouped into the following categories: early or late truncation (defined as truncating before amino acid 271 for early or after amino acid 271 for late), missense, large deletion, individually as 1 of the 8 common mutations (R106W, R133C, T158M, R168X,

R255X, R270X, R294X, R306C), no mutation with expanded testing, or unknown. BMI was calculated from height and weight measurements and converted to sex- and age- appropriate z scores (18). BMI z scores were categorized as underweight (<-2 standard deviations [SD]), normal weight (± 2 SD), or overweight (>2 SD) relative to the reference standards. The location of the clinical and research sites included Houston, TX; Chicago, IL; Tampa/Miami, FL; New Brunswick, NJ; and Oakland, CA.

Nutritional Data

Dietary sources of vitamin D included a categorical (yes/no) assessment of the daily use of multivitamin supplements containing vitamin D, 400 IU/dose; the daily consumption of vitamin D-fortified milk, whether skim, 1%, 2%, whole milk, or soy; and the daily consumption of a standard, vitamin D-fortified, commercially available, pediatric or adult formula (eg, Pediasure, Boost Kids Essentials, Vital Jr, Peptamen Jr). The frequency of milk and formula consumption was assessed further as the number of 8-oz glasses of milk (vitamin D content ~ 100 IU/8 oz) or containers of formula (vitamin D content ~ 400 IU/8 oz) consumed daily. The categorical (yes/no) use of anticonvulsant medications was recorded, as well as the identification of specific drugs. The degree of mobility was used as a proxy for physical activity and was categorized as nonambulatory, ambulates independently, or ambulates with support. Scoliosis was recorded as present or absent.

Laboratory Data

Clinical laboratory reports were reviewed for serum 25-(OH)D and intact PTH values. Serum 25-(OH)D and intact PTH levels were measured by standard clinical radioimmunoassay and electrochemiluminescent methods, respectively (Quest Laboratories Nichols Institute, San Juan Capistrano, CA; ARUP Laboratories, Salt Lake City, UT). The seasons of the year during which laboratory samples were obtained were categorized as summer (June, July, August), fall (September, October, November), winter (December, January, February), or spring (March, April, May). Vitamin D deficiency was defined as serum 25-(OH)D concentrations <50 nmol/L (17). Vitamin D sufficiency was defined as serum 25-(OH)D concentrations ≥ 50 nmol/L.

Statistical Analysis

Descriptive statistics (MiniTab, version 11.0, MiniTab Inc, State College, PA) were expressed as mean \pm SD if normally distributed or as median with first and third interquartile ranges if not normally distributed. Categorical variables were presented as frequency distributions. Nonparametric statistics using Kruskal-Wallis tests were applied to detect differences in 25-(OH)D concentrations based on race or ethnicity, dietary intake of vitamin D food sources, nutritional (BMI) status, present use of anticonvulsants, degree of mobility, season of the year, site location, and *MECP2* status. Mann-Whitney tests were used to determine post hoc differences in 25-(OH)D and groups of dietary sources of vitamin D and race or ethnicity factors. Regression analysis was used to determine relations between serum 25-(OH)D and BMI or PTH concentrations. General linear modeling was used to determine the relation between serum 25-(OH)D concentrations and race or ethnicity, dietary factors, nutritional status, present use of anticonvulsants, degree of mobility, season of the year, and *MECP2* status combined after adjusting for age. Values were considered to be statistically significant if $P < 0.05$.

RESULTS

Subject Characteristics

The characteristics of individuals with RTT ($n = 284$) are shown in Table 1. The age range of the cohort was skewed toward younger individuals with a median (first and third interquartile) age of 9.0 (5.7–16.1) years. The racial and ethnic distribution was primarily white (non-Hispanic). The nutritional status of the cohort was predominantly normal weight-for-height based on BMI z scores. *MECP2* status was determined in 99% of the cohort; *MECP2* mutations were found in 94% of those tested. Individuals with 1 of the 8 common mutations comprised 60% of those tested.

Vitamin D Status and Related Measures

Serum 25-(OH)D levels were documented in 55% ($n = 157$) of the cohort. Serum 25-(OH)D concentrations were <75 nmol/L in 60% ($n = 95$) and <50 nmol/L in 20% ($n = 32$) of the group. The median (first and third interquartile) 25-(OH)D concentration for the cohort was 70 (52–82) nmol/L; 1 individual had a value in excess of 50 nmol/L by a factor of 7-fold because of excessive supplemental ingestion. Median 25-(OH)D concentrations differed between those individuals with levels above and below the cutoff value of 50 nmol/L (75 vs 37 nmol/L, $P < 0.001$). The majority (81%) of the 25-(OH)D samples was obtained at the Texas site; median 25-(OH)D concentrations did not differ among locations because of the small sample sizes of the remaining sites (data not shown). Nearly two-thirds of the serum 25-(OH)D samples were obtained in the fall (24% of cohort) and winter (40% of cohort) months; however, median 25-(OH)D concentrations did not differ among the seasons of the year. The median (first and third

interquartile) PTH concentration of the group ($n = 74$) was 22 ng/L (14, 31). Serum 25-(OH)D concentrations were not associated with age, BMI, or serum PTH concentrations (data not shown).

Dietary, Medication, and Ambulatory Variables

Dietary sources of vitamin D, including multivitamin supplements, vitamin D–fortified milk, and commercial formulas, were consumed by 40%, 52%, and 54%, respectively, of the cohort. The number of glasses of milk and containers of commercial formulas consumed daily was 1.5 ± 0.9 and 3.0 ± 1.7 , respectively. Fifty percent of the cohort consumed daily 1 dietary source of vitamin D, 37% consumed 2 dietary sources, 7% consumed 3 dietary sources, and 6% consumed 0 dietary sources of vitamin D. Anticonvulsant medications were used daily by 57% of the cohort; within this group ($n = 163$), 61% used 1 anticonvulsant, 31% used 2 anticonvulsants, and 8% used ≥ 3 anticonvulsants. The most commonly used anticonvulsants included sodium valproate (26%), zonisamide (24%), levetiracetam (23%), and lamotrigine (21%). Thirty-nine percent of the cohort ambulated independently, 36% was nonambulatory, and 25% ambulated with support. Scoliosis was present in 54% of the cohort.

Variables Associated With Serum 25-(OH)D Levels

Dietary vitamin D sources and race or ethnicity factors that contributed to serum 25-(OH)D concentrations are shown in Table 2. Median serum 25-(OH)D concentrations were significantly lower in individuals who did not receive multivitamin supplements or commercial formulas than in those who did. When individuals were grouped on the basis of the number of dietary sources of vitamin D consumed daily, median serum 25-(OH)D concentrations were significantly lower in the group with none versus those who consumed 1, 2, or 3 dietary sources of vitamin D. Median serum 25-(OH)D concentrations did not differ significantly based on age, nutritional status as measured by BMI z scores, consumption of vitamin D–fortified milk, present use of anticonvulsants, ambulatory status, presence of scoliosis, season of the year, clinic or research site location, or *MECP2* status (data not shown). Median serum 25-(OH)D concentrations were significantly different among the racial and ethnic groups, but the number of individuals in some minority groups was small. Post hoc analyses demonstrated significant differences in median serum 25-(OH)D levels between white and Hispanic, but not white and African American girls and women.

When all of the variables, including race and ethnicity, nutritional status, *MECP2* mutation, dietary consumption of multivitamins, milk, or formula, use of anticonvulsant medications, ambulatory status, presence of scoliosis, and season of the year for blood sampling, were combined and adjusted for age, only the consumption of multivitamin supplements predicted differences in serum 25-(OH)D concentrations; values were higher in those girls and women who consumed multivitamin supplements compared with those who did not (83 ± 60 vs 62 ± 52 nmol/L, $P < 0.01$).

DISCUSSION

Low bone mineral density and increased risk of bone fractures characterize the natural history of bone health in RTT (6). Our study demonstrated that vitamin D deficiency, that is, 25-(OH)D levels <50 nmol/L, was present in 20% of affected girls and women who were tested. The use of vitamin supplements or vitamin D–fortified formula favorably influenced serum 25-(OH)D concentrations, whereas the use of anticonvulsants did not adversely affect

TABLE 1. Characteristics of girls and women ($n = 284$) with Rett syndrome

Characteristic	Value*
Age, y	11.7 ± 8.4
Race/ethnicity	
White (% of cohort)	69
African American (% of cohort)	5
Hispanic (% of cohort)	19
Asian/Pacific Islander (% of cohort)	5
Native American (% of cohort)	2
BMI z score	-0.4 ± 1.7
Nutritional status†	
Underweight (BMI z score < -2) (% of cohort)	15
Normal weight (BMI z score -2 – 2) (% of cohort)	81
Overweight (BMI z score > 2) (% of cohort)	4
<i>MECP2</i> mutation	
Missense (% of cohort)	29
Early truncation (% of cohort)	35
Late truncation (% of cohort)	15
Large deletion (% of cohort)	12
Negative with expanded testing (% of cohort)	6
Unknown (% of cohort)	3

BMI = body mass index; *MECP2* = methyl-CpG-binding protein 2; SD = standard deviation.

*Values expressed % of cohort for group variables and as mean \pm SD for continuous variables.

†Nutritional status expressed as BMI.

TABLE 2. Dietary sources of vitamin D and race or ethnicity factors that contribute to serum 25-hydroxyvitamin D concentrations in girls and women with Rett syndrome (n = 157)

Variable	No. subjects	Median 25-(OH)D, nmol/L	P
Multivitamin use			
No	103	65	<0.05
Yes	54	75	
Milk consumption			
No	81	72	NS
Yes	67	65	
Commercial formula use			
No	62	60	<0.001
Yes	95	72	
No. dietary sources of vitamin D consumed daily			
0	13	45	—*
1	82	70	<0.001
2	52	66	<0.001
3	10	76	<0.001
Race and ethnicity			
White	99	72	—*
African American	10	54	NS
Hispanic	40	59	<0.001
Asian	4	57	—
Native American	4	79	—

25-(OH)D = 25-hydroxyvitamin D; NS = not significant.

* Post hoc Mann-Whitney tests for 0 vs 1, 2, or 3 dietary sources of vitamin D and white vs African American or Hispanic groups; Asian and Native American groups not tested because of small sample size.

their vitamin D status. We speculate that attention to vitamin D nutriture may favor enhanced bone mineral density and reduce the frequency of bone fractures in girls and women with RTT (14).

Evidence from single-center studies suggests that vitamin D deficiency occurs in healthy children of all ages (19–21). In a nationally representative sample of US children ages 1 to 11 years, 15% had values <50 nmol/L (22). Among healthy adolescents, 42% were vitamin D deficient (≤ 50 nmol/L) (19). Low serum vitamin D levels are found in children with a variety of chronic diseases (23–27). Twenty-one percent of children with metabolic bone disease associated with chronic diseases had vitamin D levels <50 nmol/L (23). The prevalence of vitamin D deficiency in the RTT cohort approximated values found in unaffected healthy children as well as some but not all individuals with other chronic disorders. Although our study did not include a control group, our data support the observation that vitamin D deficiency is not unique to RTT. Thus, early screening for vitamin D deficiency may be an important component of the health care of girls and women with RTT. The somewhat lower prevalence may be related to the findings that the majority of the RTT cohort was well-nourished by BMI criteria and 40% received a supplement that reliably provided adequate dietary intakes of vitamin D.

The cutoff value for vitamin D deficiency in children is controversial because available evidence is not sufficient to support the use of bone mineral content or PTH concentrations as functional outcomes in these individuals (11,21). Several groups have defined vitamin D deficiency in children as a serum 25-(OH)D level <37.5 nmol/L and vitamin D sufficiency as >50 nmol/L (9,21). In adults, vitamin D deficiency, insufficiency, and sufficiency have been defined as 25-(OH)D levels <45 nmol/L, ≥ 45 to <75 nmol/L, and ≥ 75 nmol/L, respectively, based on all-cause mortality and functional biomarkers including the serum PTH response to serum

25-(OH)D levels, calcium absorption (balance) studies, and bone mineral content, measured by dual-energy absorptiometry (28–31). In addition, serum 25-(OH)D levels ≥ 100 nmol/L are associated with maximal suppression of PTH and reduced fracture rates in adults (14). If we used these higher cutoff values, the prevalence of vitamin D deficiency and insufficiency in the RTT cohort would have been 15% and 45%, respectively. In children, serum 25-(OH)D concentrations may be inversely associated with serum PTH concentrations, but the correlation is poor (19,21,26). We did not observe a relation between serum 25-(OH)D and PTH concentrations in our RTT cohort; however, only 26% had serum PTH levels measured.

Although many factors contribute to vitamin D deficiency, our study was neither designed nor powered to address the significance of risk factors on serum vitamin D status. A higher prevalence of vitamin D deficiency has been identified in older than in younger children (20,22). Low serum 25-(OH)D levels may be inversely associated with body fatness (19,20,32). In the present study, we did not show age- or BMI-related differences in serum 25-(OH)D concentrations in the RTT cohort. Decreased vitamin D synthesis in healthy children may be associated with decreased sunlight exposure because of inherently dark skin, the use of sunscreen, an indoor lifestyle, the latitude at which they lived, or the season of the year (19–22,24,26,32). Of all of these factors, only race and ethnicity influenced 25-(OH)D levels in the RTT cohort. An increased prevalence of low 25-(OH)D concentrations has been reported in healthy African American and Hispanic adolescents, compared with healthy white individuals in some but not all studies (19–23,32,33). We also found lower 25-(OH)D concentrations in Hispanic but not African American girls and women with RTT, but our study was limited by an insufficient number of individuals in some of the racial and ethnic groups to achieve statistical power to

detect significance. We used degree of mobility as a surrogate marker of physical activity, and hence exposure to sunlight; however, the ambulatory status of girls and women with RTT did not show an association with 25-(OH)D concentrations. Anecdotal reports from parents suggest that most girls with RTT have limited outdoor sunlight exposure. Furthermore, children in southern latitudes are less likely to be outside during the intense summer heat. These observations may explain in part the lack of seasonal and site variation of 25-(OH)D levels in the RTT cohort, although our study was not powered to detect differences among the groups. Finally, genetic factors may influence serum 25-(OH)D levels. Differences in vitamin D-binding protein genotypes, particularly T436K, may explain in part the variability of serum 25-(OH)D levels among groups of individuals (34). We did not characterize vitamin D-binding protein in the present study.

Decreased dietary intake of vitamin D-fortified foods, beverages, or supplements may contribute to vitamin D deficiency (12,19–22,24). Our data demonstrated that girls and women with RTT who did not consume vitamin D-fortified milk, formula, or supplements had quantitatively lower 25-(OH)D concentrations than those who consumed ≥ 1 dietary sources of vitamin D. Vitamin supplements and formula consumption provided the strongest association with serum 25-(OH)D concentrations because they contributed on average 400 and 300 IU, respectively, of dietary vitamin D daily. Limited milk consumption, that is, an average of 1.5 glasses daily, failed to influence serum vitamin D levels in the RTT cohort because this amount provided only 150 IU of dietary vitamin D daily. When all of the factors were combined, the use of multivitamin supplements best predicted favorable 25-(OH)D concentrations. In children with inflammatory bowel disease, 25-(OH)D concentrations were 31% higher in those who received a multivitamin supplement than in those who did not (24); in the RTT cohort, 25-(OH)D concentrations were only 15% higher in those girls and women who received a multivitamin supplement. In a 2009 report, only 6% of healthy children who received a multivitamin supplement containing 400 IU of vitamin D had serum 25-(OH)D concentrations < 50 nmol/L (22). Our data showed that 22% of individuals who received a multivitamin supplement and were tested for their vitamin status had 25-(OH)D values < 50 nmol/L. These observations suggest that dietary vitamin D requirements for girls with RTT may be higher than 400 IU/day, assuming that parents were consistent in the administration of the multivitamin supplement.

The use of medications such as anticonvulsants is associated with vitamin D deficiency (35). A variety of older anticonvulsants that induce the hepatic CYP₄₅₀ enzyme system, including phenobarbital, phenytoin, and carbamazepine, have been implicated (36); however, newer anticonvulsants may equally affect the vitamin D status of individuals with seizure disorders (37). A direct effect of the drugs on bone cells has been proposed, but the effect of the newer-generation antiepileptic medications on bone metabolism is unknown. Although $> 50\%$ of the RTT cohort received anticonvulsants, we did not find an association between their use and 25-(OH)D concentrations, but we did not determine whether the dose or duration of use differed among individuals. We observed that 85% of the RTT cohort received the newer anticonvulsants.

The prevention and treatment of vitamin D deficiency can be accomplished by greater exposure to sunlight in the absence of sunscreen for adequate vitamin D synthesis and the consumption of vitamin D-fortified beverages or use of multivitamin supplements. Exposure of 20% of the unclothed body surface to the midday sun for 10 to 15 minutes in spring, summer, and fall is considered adequate for light-skinned people, providing approximately 25% of the amount of ultraviolet light required to produce slight pinkness of the skin (38). Dark-skinned individuals require 6 to 10 times as much ultraviolet light exposure as light-skinned individuals to

achieve equivalent vitamin D concentrations (9). Children who do not ingest daily 1 L of vitamin D-fortified milk should receive a supplement of at least 400 IU/day, with additional considerations for skin color and home latitude (10). Children with increased risk for vitamin D deficiency may require higher doses of vitamin D supplementation to achieve serum 25-(OH)D levels ≥ 50 nmol/L. Variability in vitamin D-binding protein genotypes may also predict serum 25-(OH)D responses to supplementation (34). Because of the increased risk of low bone mineral density and fractures in girls and women with RTT (6), we routinely assess dietary vitamin D intakes, query the amount of sunlight exposure, determine serum 25-(OH)D concentrations, and implement oral therapy if necessary. The long-term benefit of this practice on bone health remains to be determined.

In summary, vitamin D deficiency is prevalent in girls and women with RTT. The use of multivitamin supplements or commercial formulas is associated with improved vitamin D status in these individuals. Attention to vitamin D nutrition may improve bone health in this disorder.

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