Research and Professional Briefs

High Risk of Vitamin D Deficiency in Children with Sickle Cell Disease

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ABSTRACT

Vitamin D is a particularly concerning nutrient for children with homozygous SS sickle cell disease (SCD-SS) due to their increased skin melanin concentrations, reduced levels of physical activity, and poor vitamin D intake. The goal of this study was to compare the vitamin D status of children with SCD-SS to healthy African-American children living in the same geographic area. Growth, dietary intake, serum 25-hydroxyvitamin D [25(OH)D], and intact parathyroid hormone (iPTH) concentrations were measured in 61 African-American subjects with SCD-SS and 89 healthy African-American control subjects age 5 to 18 years from the Philadelphia, PA, region (latitude 39.95° N). Median serum 25(OH)D concentrations were 15 ng/mL (95% confidence interval [CI]: 13, 17) in subjects with SCD-SS and 21 ng/mL (95% CI: 18, 22) in healthy control subjects ($P<0.0002$). Vitamin D deficiency [25(OH)D<11 mg/mL] was found in 33% of subjects with SCD-SS and 9% of healthy control subjects ($P<0.001$); 25% of subjects with SCD-SS and 17% of healthy control subjects had elevated iPTH [(>59 ρg/mL), $P<0.05$]. Ninety-three percent of subjects with SCD-SS and 90% of healthy subjects had vitamin D insufficiency [25(OH)D<30 mg/mL]. The risk of vitamin D deficiency among subjects with SCD-SS was 5.3 (95% CI: 2.5, 8.2) times greater than control subjects, adjusted for season and age. Poor vitamin D status was prevalent in children with SCD-SS and healthy African-American children living in the same geographic area. However, children with SCD-SS were at greater risk for vitamin D deficiency than healthy African-American children.

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Sickle cell disease (SCD) is a hereditary disorder affecting primarily Africans and African Americans (1). Children homozygous for the SS allele (SCD-SS) are the most severely affected and are at risk for nutrient deficiencies due to decreased appetite (2), poor dietary intake (3,4), and increased resting energy expenditure (5,6). Vitamin D is particularly concerning for children with SCD-SS due to their increased skin melanin concentrations, reduced levels of physical activity (6), and poor vitamin D intake (4,7). Low bone mineral density in children with SCD (8,9) is further cause for concern about the possible consequences of vitamin D deficiency.

The best clinical indicator of vitamin D status is serum 25-hydroxyvitamin D [25(OH)D] concentration, which represents the summation of cutaneous vitamin D synthesis and ingestion of either vitamin D-2 (ergocalciferol) or vitamin D-3 (cholecalciferol) (10). There are no universally accepted definitions of vitamin D deficiency and insufficiency; however, the Dietary Reference Intakes define vitamin D deficiency as 25(OH)D concentrations less than 11 ng/mL (10). A review article of research in adults concluded that the most advantageous 25(OH)D concentrations for bone mineral density and other health outcomes began at 30 ng/mL (11). Similar data are not available in children.

Recent studies suggest that vitamin D insufficiency is common in healthy children in the United States (12-15). Predictors of vitamin D status include: ethnicity, season, age, body mass index (BMI), use of dietary supplements, and milk consumption (12,14). Factors influencing the cutaneous synthesis of vitamin D include latitude, time of day, skin pigmentation, and the amount of skin exposed (10,16,17). Reports of low 25(OH)D concentrations in healthy children underscore the importance of a control group of similar ethnic composition from the same geographic region and of controlling for season when evaluating vitamin D status in children with chronic diseases.

The purpose of this study was to compare the vitamin D status of children with SCD-SS with a healthy, African-

American reference group living in the same geographic area.

METHODS

Study Design and Participants

Children with SCD-SS, ages 5 to 18 years, were recruited from the Comprehensive Sickle Cell Center at The Children's Hospital of Philadelphia, Philadelphia, PA (latitude 39.95°N). Participants were recruited by letters, phone calls, and in person during routine clinic visits. In addition, information about the study was included in several of the Sickle Cell Center's family newsletters. Inclusion criteria were: no transfusions in the previous two months and liver enzymes within twice the normal range. Exclusion criteria included: other medical conditions known to affect growth, dietary intake or nutritional status; chronic transfusion therapy; history of stroke; hydroxyurea therapy; and weight or height greater than the 97th percentile for age and sex (2000 National Center for Health Statistics/Centers for Disease Control and Prevention growth charts) (18). Subjects participated when they were in a steady state of health and had not experienced a fever or pain event for at least 2 weeks.

Healthy African-American children ages 6 to 18 years were recruited through newspaper advertisements, mailings, and fliers to primary care centers and pediatric practices affiliated with The Children's Hospital of Philadelphia and the surrounding community. Exclusion criteria were any disease or use of medication known to affect growth, nutritional status, or bone health; or reported weight or height more than the 95th percentile or less than the 5th percentile (18).

Informed consent was obtained from the parent/guardian of each subject and assent was obtained from children ages 7 years and older. Participants were compensated with monetary reimbursement and free parking for their time and provided with a small thank you gift. The Institutional Review Board of The Children's Hospital of Philadelphia approved the protocol.

Anthropometry

Weight and height were measured using a digital scale accurate to 0.1 kg (Scaltronix, White Plains, NY) and a stadiometer accurate to 0.1 cm (Holtain, Crymych, UK). BMI was calculated. Height, weight, and BMI were compared with the National Center for Health Statistics/Centers for Disease Control and Prevention 2000 reference standards (18).

Laboratory Measures

A fasting blood sample was obtained in the SCD-SS group and a nonfasting sample was obtained in the control group. No evidence exists to support diurnal or postprandial changes in 25(OH)D concentrations. Serum samples were stored at –70° C before analysis of 25(OH)D and iPTH (Quest Nichols Laboratory, San Juan Capistrano, CA). Serum 25(OH)D was analyzed by ^{125}I-labeled radioimmunoassay using a commercially available test kit (DiaSorin, Inc, Stillwater, MN) (19). The DiaSorin primary antibody demonstrates equal reactivity with 25(OH)D-2 and 25(OH)D-3 and has an excellent correlation with high-performance liquid chromatography (HPLC) (20). The intra- and inter-assay precisions (% coefficient of variation [CV]) were 2.2% to 8.6%. Serum intact parathyroid hormone (iPTH) was measured by the immunochemiluminometric assay (Quest Nichols Laboratory) with a sensitivity of 1 pg/mL (interassay CV, 7% to 9%). In subjects with SCD-SS, blood was analyzed immediately for alanine transaminase, aspartate transaminase, gamma glutamyl transpeptidase, and bilirubin, in the clinical laboratory.

Dietary Intake

Dietary intake was estimated by three 24-hour recalls (2 weekdays and 1 weekend day). Diet records were analyzed using Minnesota Nutrition Data System Version 2006 (Minneapolis, MN). Vitamin D and calcium intakes were compared to the Adequate Intakes (AI) (10). The usage and type of vitamin and mineral supplements were obtained by questionnaire.

Statistical Analysis

Vitamin D deficiency was defined as 25(OH)D concentration less than 11 ng/mL (10), vitamin D insufficiency as less than 30 ng/mL to greater than or equal to 11 ng/mL (11), and vitamin D sufficiency as 30 ng/mL or more. Vitamin D concentrations were reported by season: winter (December, January, February), spring (March, April, May), summer (June, July, August), and autumn (September, October, November). The reference range from Quest Nichols Laboratory for iPTH concentrations was 9 to 59 ρg/mL. The Student t test or Wilcoxon-Mann-Whitney test was used to assess group differences. χ^2 test was used to assess differences in proportions. Logistic regression analysis was used to determine the risk for vitamin D deficiency in subjects with SCD-SS compared with healthy children, after adjusting for season and age. Because vitamin D deficiency was common, the odds ratio was adjusted for frequent outcomes to avoid overestimation of the relative risk (21).

The relationship between 25(OH)D and iPTH concentrations was analyzed by correlation. Calcium and vitamin D intakes were compared to the AI and reported as a percentage of the AI (%AI) (10). This study had a 90% power to detect a 25% difference in the proportion of vitamin D deficiency between the groups. Statistical significance was defined as $P<0.05$. Statistical analyses were performed using STATA 9.0 (StataCorp, College Station, TX).

RESULTS AND DISCUSSION

Sixty-one subjects with SCD-SS (10.5±3.4 years, 49% females) and 89 healthy African-American subjects (10.9±2.6, 46% females) participated. Children with SCD-SS had a lower BMI than the healthy subjects (16.1±2.8 vs 19.5±4.1, $P<0.0001$).

The median serum 25(OH)D concentrations were 15 ng/mL (interquartile range [IQR],10 to 33) in the subjects with SCD-SS and 21 ng/mL (IQR, 15 to 36) in the healthy control subjects ($P<0.0002$). There were seasonal fluctuations in 25(OH)D concentrations in both groups and

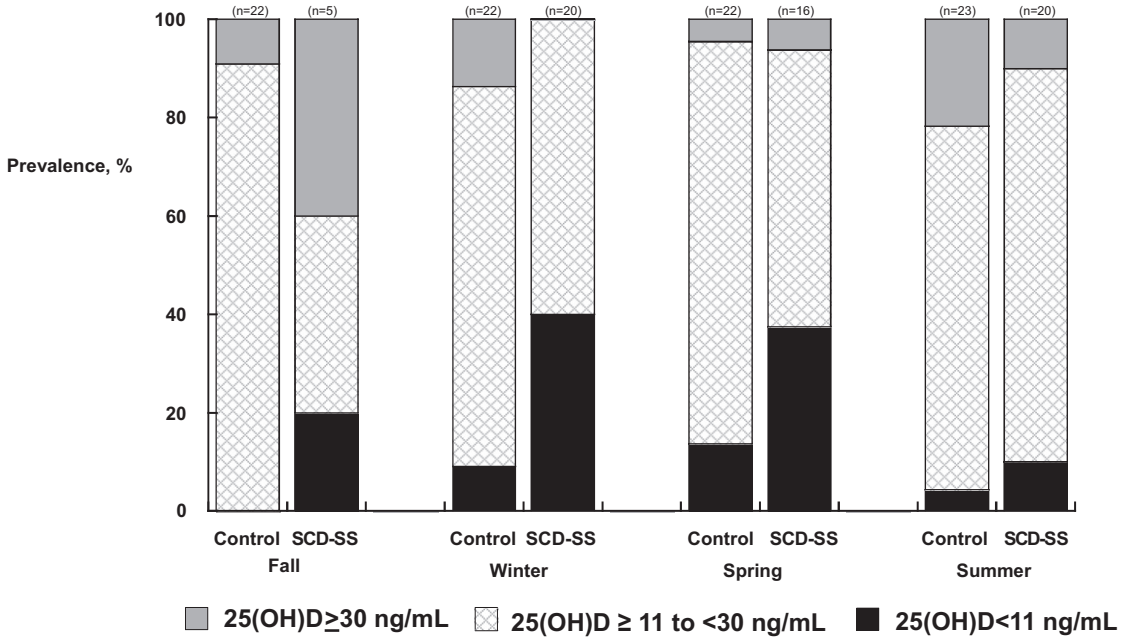

Figure 1. Season and group differences in the prevalence of vitamin D deficiency and insufficiency among subjects with sickle cell disease type SS (SCD-SS) and healthy African-American control subjects. Seasons were categorized as winter (December, January, February), spring (March, April, May), summer (June, July, August), and autumn (September, October, November). Vitamin D deficiency was defined as <11 ng/mL (10), vitamin D insufficiency as ≥11 to <30 ng/mL (11), and vitamin D sufficiency as ≥30 ng/mL.

during every season 25(OH)D concentrations were lower in the SCD-SS group compared to the healthy controls. Twenty subjects with SCD-SS (33%) and eight (9%) healthy subjects were vitamin D deficient ($P<0.001$). Ninety-three percent of subjects with SCD-SS and 90% of healthy subjects had vitamin D insufficiency. Figure 1 presents seasonal distributions of serum 25(OH)D concentrations.

In the subjects with SCD-SS, 25(OH)D concentrations were negatively associated with age ($r=-0.41$, $P<0.0009$) and alanine transaminase ($r=-0.50$, $P<0.0002$), and were positively associated with vitamin D intake ($r=0.44$, $P<0.002$). In the healthy subjects, 25(OH)D concentrations were negatively associated with age ($r=-0.26$, $P<0.02$) and BMI z score ($r=-0.22$, $P<0.04$), but not with dietary intake.

Logistic regression analysis indicated that the risk for vitamin D deficiency among subjects with SCD-SS was 5.3 (95% confidence interval: 2.5, 8.2) times greater than among the controls after adjusting for season and age.

Subjects with SCD-SS consumed 69% AI for vitamin D and 60% AI for calcium. Healthy subjects consumed 74% AI for vitamin D and 57% AI for calcium. None of the subjects with SCD-SS took a supplement containing vitamin D or calcium. Ten percent of the healthy subjects were taking a supplement that contained vitamin D (median, 50 IU; IQR, 0 to 400 IU) and calcium (median, 100 mg; IQR, 0 to 200 mg).

Vitamin D was examined in relation to iPTH (Figure 2). In children with SCD-SS there was a statistically significant correlation between 25(OH)D and iPTH concentrations ($r=-0.35$, $P<0.006$). There was a weaker correla-

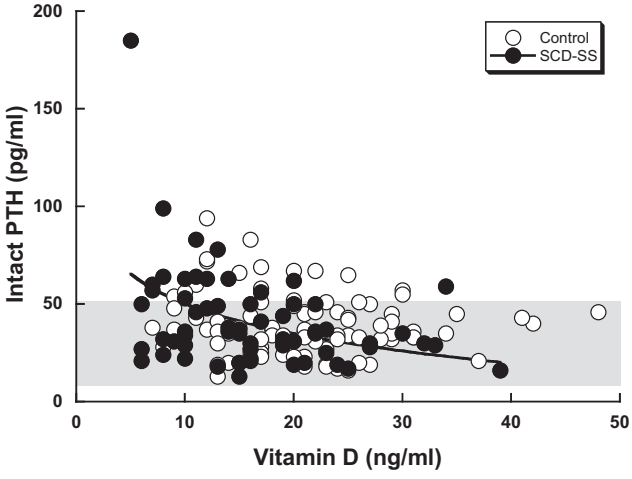

Figure 2. Vitamin D and intact parathyroid hormone (iPTH) concentrations in subjects with sickle cell disease type SS (SCD-SS) and healthy African-American control subjects. The closed circles represent subjects with SCD-SS, and the open circles represent the healthy control subjects. Vitamin D deficiency was defined as 25(OH)D<11 ng/mL, insufficiency as ≥11 to <30 ng/mL, and sufficiency as ≥30 ng/mL. The normal range for iPTH is between 9 and 52 ρg/mL (gray shaded area). In children with SCD-SS, there was a statistically significant correlation between 25(OH)D and iPTH concentrations ($r=-0.35$, $P<0.006$). There was no correlation between 25(OH)D concentrations and iPTH in healthy children ($r=-0.13$). To convert 25(OH)D to nanomoles per liter, multiply by 2.5.

tion between 25(OH)D concentrations and iPTH concentrations in healthy children ($r=-0.13$, $P<0.05$). Twenty-five percent of subjects with SCD-SS (n=15) and 17% of healthy subjects (n=15) had elevated iPTH concentrations ($P<0.05$).

This is the first study of vitamin D status in children with SCD-SS that included a wide age range as well as an appropriate reference group of similar ethnicity. It demonstrated that 25(OH)D concentrations in children with SCD-SS were significantly lower compared with healthy African-American children from the same geographic area, who also had poor vitamin D status. After adjusting for season and age, vitamin D deficiency was 5.3 times greater in children with SCD-SS than in healthy African-American children. Vitamin D deficiency occurred in 33% of subjects with SCD-SS and 9% of healthy control subjects. Apparent secondary hyperparathyroidism was observed in the SCD-SS group, with an elevated iPTH concentration correlating with low 25(OH)D concentrations.

These findings are consistent with results from two previous studies of low 25(OH)D concentrations in SCD-SS (7,9). In a study by Lal and colleagues, vitamin D status was low (<27.5 nmol/L=11 ng/mL) in 30% of the 25 children (9 to 19 years) with SCD-SS and severe disease manifestations in Oakland, CA (latitude 37.80° N) (9). The similar rates of vitamin D deficiency in the current study in Philadelphia, PA (latitude 39.95° N) compared with the study in California was somewhat surprising. There are several possible explanations for these findings. Most children with SCD-SS are African-American and have increased skin melanin concentrations, so regardless of geographic location, they may not spend adequate time outdoors to synthesize enough vitamin D. Other possibilities are that children with SCD-SS have higher vitamin D requirements or impaired vitamin D metabolism. In a second investigation, Buison and colleagues reported low vitamin D status in 65 children (5 to 18 years old) with SCD-SS in Philadelphia, PA. The mean 25(OH)D concentration was 25.5 nmol/L (11.6 ng/mL), and 65% of the subjects were vitamin D–deficient (7). In that study, a healthy comparison group was only available for a subset of children, ages 7 to 10 years of age. In this limited comparison, 25(OH)D concentrations were lower in children with SCD-SS compared with healthy African-American children in all seasons. PTH concentrations were also higher in children with SCD-SS compared with healthy children. The current study from a different sample of children with SCD-SS in a wider age range and with an appropriate healthy reference group confirms Buison and colleagues' findings of low vitamin D status and elevated PTH in children with SCD-SS. Studies in healthy children have demonstrated an age-related decrease in vitamin D status (14); therefore, including children with SCD-SS in this study up to an age of 18 years provided a better estimate of vitamin D status throughout childhood.

No vitamin D supplementation study has been conducted in children with SCD-SS. In healthy children, two randomized, placebo-controlled vitamin D supplementation studies of healthy girls demonstrated increased bone mineral content, decreased bone resorption, and increased lean body mass (22,23). A randomized, controlled study of vitamin D supplementation is required to determine whether optimizing vitamin D concentrations positively influences bone mineral content or body composition in children with SCD-SS.

CONCLUSIONS

Our study demonstrated that children with SCD-SS had low 25(OH)D concentrations and dietary intake of calcium and vitamin D that was well below the AI. Health care professionals treating children with SCD-SS should be aware of this and educate families about ways to improve their children's intakes of these important nutrients. In addition, for those with vitamin D deficiency, supplementation is likely required because it would be virtually impossible to adequately increase such low 25(OH)D concentrations with food sources alone.

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References

1. Fixler J, Styles L. Sickle cell disease. *Pediatr Clin North Am*. 2002; 49:1193-1210.
2. Mitchell MJ, Kawchak DA, Stark LJ, Zemel BS, Ohene-Frempong K, Stallings VA. Brief report: parent perspectives of nutritional status and mealtime behaviors in children with sickle cell disease. *J Pediatr Psychol*. 2004;29:315-320.
3. Williams R, George EO, Wang W. Nutrition assessment in children with sickle cell disease. *J Assoc Acad Minor Phys*. 1997;8:44-48.
4. Kawchak DA, Schall JI, Zemel BS, Ohene-Frempong K, Stallings VA. Adequacy of dietary intake declines with age in children with sickle cell disease. *J Am Diet Assoc*. 2007;107:843-848.
5. Barden EM, Zemel BS, Kawchak DA, Goran MI, Ohene-Frempong K, Stallings VA. Total and resting energy expenditure in children with sickle cell disease. *J Pediatr*. 2000;136:73-79.
6. Buchowski MS, Townsend DW, Williams R, Chen KY. Patterns and energy expenditure of free-living physical activity in adolescents with sickle cell anemia. *J Pediatr*. 2002;140:92.
7. Buison AM, Kawchak DA, Schall J, Ohene-Frempong K, Stallings VA, Zemel BS. Low vitamin D status in children with sickle cell disease. *J Pediatr*. 2004;145:622-627.
8. Buison AM, Kawchak DA, Schall JI, Ohene-Frempong K, Stallings VA, Leonard MB, Zemel BS. Bone area and bone mineral content deficits in children with sickle cell disease. *Pediatrics*. 2005;116:943-949.
9. Lal A, Fung EB, Pakbaz Z, Hackney-Stephens E, Vichinsky EP. Bone mineral density in children with sickle cell anemia. *Pediatr Blood Cancer*. 2006;47:901-906.
10. Institute of Medicine. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. Washington, DC: National Academies Press; 1997:1-432.
11. Bischoff-Ferrari HA, Giovannucci E, Willet WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hy-

droxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006; 84:18-28.
12. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone*. 2002;30:771-777.
13. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med*. 2004;158:531-537.
14. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS. Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. *Am J Clin Nutr*. 2007;86:150-158.
15. Sullivan SS, Rosen CJ, Halteman WA, Chen TC, Holick MF. Adolescent girls in Maine are at risk for vitamin D insufficiency. *J Am Diet Assoc*. 2005;105:971-974.
16. Holick MF. Vitamin D: The underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes*. 2002;9:87-98.
17. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab*. 1988;67:373-378.
18. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL. *CDC Growth Charts: United States*. 6-8-2000. US Department of Health and Human Services. Washington, DC: Advance Data 3141-3127.
19. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. *Clin Chem*. 1993;39:529-533.
20. Hollis BW. Comparison of commercially available (125)I-based RIA methods for the determination of circulating 25-hydroxyvitamin D. *Clin Chem*. 2000;46:1657-1661.
21. Zhang J, Yu K. What's the Relative Risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA*. 1998;280: 1690-1691.
22. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R. Effect of vitamin D replacement on musculoskeletal parameters in school children: A randomized controlled trial. *J Clin Endocrinol Metab*. 2006;91:405-412.
23. Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Mølgaard C, Lamberg-Allardt C. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: A double-blinded randomized placebo-controlled 1-year intervention. *J Bone Miner Res*. 2006;21:836-844.