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## Vitamin D insufficiency in steroid-sensitive nephrotic syndrome in remission

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**Abstract** Serum 25-hydroxyvitamin D [25(OH)D] concentrations are the best indicator of vitamin D nutritional status. We measured serum 25(OH)D concentrations in 94 healthy controls and in 41 subjects (aged 4–22 years) with steroid-sensitive nephrotic syndrome (SSNS) in remission. Children with remitted SSNS had significantly lower 25(OH)D concentrations than healthy controls (median 16.4 ng/ml versus 23.9 ng/ml,  $P < 0.001$ ). In a multivariable logistic regression model, the odds ratios (OR) of vitamin D insufficiency [25(OH)D  $< 20$  ng/ml] were independently increased in SSNS subjects [OR 11.2 (95% confidence interval 3.5–36.2)], non-whites [OR 12.9 (4.6–36.2)], older children [OR 1.20 per year (1.06–1.36)], and winter months [OR 6.7 (2.5–18.4)]. Within the SSNS subjects, multiple linear regression determined that serum 25(OH)D concentrations were not associated with SSNS disease characteristics measured in this study, such as duration of disease, number of relapses, cumulative glucocorticoids, and interval since last relapse. In conclusion, children with remitted SSNS have lower serum 25(OH)D concentrations than healthy controls. This difference

persisted after adjusting for the potential confounding effects of age, race, season, and milk intake. Children with remitted SSNS may benefit from routine measurement of 25(OH)D, but the clinical significance of low 25(OH)D in this population remains unclear.

**Keywords** Vitamin D · 25-Hydroxyvitamin D · Nephrotic syndrome

### Introduction

Children with idiopathic nephrotic syndrome (NS) and preserved renal function often exhibit abnormal vitamin D metabolism [1]. Serum concentrations of 1,25-dihydroxyvitamin D [calcitriol or 1,25(OH)<sub>2</sub>D], the active metabolite of vitamin D, are decreased [2, 3] or in the normal range [4, 5, 6]. More importantly, serum concentrations of 25-hydroxyvitamin D [calcidiol or 25(OH)D], the best marker of body vitamin D stores and overall vitamin D nutritional status [7], are decreased in children with NS [2, 3, 4, 5, 6]. These low 25(OH)D concentrations stem from urinary excretion of vitamin D-binding protein, the carrier protein for 25(OH)D [8]. In NS, suboptimal 25(OH)D concentrations may lead to secondary hyperparathyroidism [9] and metabolic bone disease, notably osteomalacia [10, 11, 12]. A recent case series reported altered bone histology in eight children with NS and normal glomerular filtration rate (GFR), five of whom had serum 25(OH)D less than 30 ng/ml [13]. Although the authors attributed the histological changes to NS disease activity and glucocorticoid therapy [13], suboptimal 25(OH)D concentrations may have contributed to some of the changes.

Most studies of NS have examined vitamin D metabolism during periods of heavy proteinuria [2, 3, 4], but children with NS typically respond quickly and completely to high-dose glucocorticoids [14]. As a result, children with steroid-sensitive nephrotic syndrome (SSNS) are only intermittently proteinuric, during either their first attack or their relapses. During remissions, se-

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rum 25(OH)D concentrations are higher than the 25(OH)D concentrations during urinary relapses [5] and are reportedly comparable to the levels in healthy children [6]. Consequently, concern regarding vitamin D nutritional status in childhood NS has focused upon treatment-resistant NS, with its long-standing proteinuria, rather than upon SSNS [15].

Current guidelines from the National Kidney Foundation (NKF) concerning bone metabolism are lacking regarding the measurement of 25(OH)D in persons with NS and normal GFR [16]. In persons with elevated parathyroid hormone (PTH) levels and GFR below 60 ml/min per 1.73 m<sup>2</sup>, however, the NKF recommends routine measurement of serum 25(OH)D and supplementation with ergocalciferol (vitamin D<sub>2</sub>) when serum 25(OH)D is less than 30 ng/ml [16]. Before children with NS can be considered candidates for routine 25(OH)D screening, the prevalence of suboptimal 25(OH)D concentrations in this population should be ascertained.

The objective of this study was to assess the vitamin D nutritional status, as measured by serum 25(OH)D, in children and adolescents with remitted SSNS, compared with healthy controls. Our hypothesis was that patients with remitted SSNS are at increased risk for vitamin D insufficiency, which was defined as a serum 25(OH)D concentration <20 ng/ml.

## Materials and methods

### Study design and subjects

A cross-sectional study was performed to compare children with remitted SSNS and healthy controls. The protocol was approved by the Human Subjects Institutional Review Board at The Children's Hospital of Philadelphia, and all subjects and parents provided written informed consent.

Children with SSNS were identified through a systematic review of all clinic charts for patients seen over the previous 3 years in the nephrology clinics at The Children's Hospital of Philadelphia and St. Christopher's Hospital for Children, both in Philadelphia (Pa., USA). Subjects fulfilling the International Study of Kidney Disease in Children criteria for the diagnosis of SSNS [17] (negative or trace protein by urine dipstick within 8 weeks of initial glucocorticoid treatment) were eligible, provided they had had a documented relapse within the 12 months prior to the study visit. Subjects were excluded if they had (1) GFR <80 ml/min per 1.73 m<sup>2</sup> as estimated by the Schwartz formula [18] or (2) other chronic medical conditions or medications, unrelated to the nephrotic syndrome, that could potentially affect bone health or vitamin D status. Study visits were scheduled at least 14 days after urinary remission of the most recent relapse. Negative or trace protein by urine dipstick was documented at the time of the study visit.

Healthy controls were recruited from the general pediatric clinics and from the surrounding community, using newspaper advertisements and flyers. Subjects with chronic medical conditions or medications potentially affecting growth, pubertal development, nutritional status, or dietary intake were excluded. Controls were required to be between the 3rd and 97th percentiles for height and body mass index (BMI) for age and gender.

### Measurements and assays

SSNS and control subjects were evaluated in the Nutrition and Growth Laboratory at the Children's Hospital of Philadelphia, where blood was drawn and anthropometric measurements were performed. Weight (kg) was measured using a digital electronic stand-on scale and height (cm) was measured using a wall-mounted stadiometer. Age- and gender-specific standard deviation scores (z-scores) for height, weight, and BMI were calculated using the Centers for Disease Control and Prevention growth charts [19].

Circulating 25(OH)D and 1,25(OH)<sub>2</sub>D concentrations were measured by radioimmunoassay with <sup>125</sup>I-labeled tracers [20, 21]. Intact PTH was measured in the SSNS subjects only, using a second-generation immunoradiometric assay [22]. The normal range for intact PTH was 10–65 pg/ml. These assays were performed in the laboratory of Dr. Bruce W. Hollis (Medical University of South Carolina, Charleston, S.C., USA).

Subjects completed a food frequency questionnaire at the time of the study visit. Participants were asked to estimate average milk intake per day over the previous 28 days, including milk with cereal, chocolate milk, hot chocolate made from milk, and Lactaid milk.

### NS disease characteristics

The inpatient and outpatient medical charts of the SSNS children were reviewed for date of diagnosis of SSNS, total number of relapses, date of last relapse, renal biopsy results, and prior steroid-sparing therapy with alkylating agents (chlorambucil, cyclophosphamide) or cyclosporine. All doses of prednisone and methylprednisolone in the interval between the date of diagnosis and the study visit were documented. Methylprednisolone doses were converted to prednisone equivalents, and total glucocorticoid exposure was summarized as the cumulative milligrams and milligrams per kilogram of prednisone equivalents between the first dose and the last dose.

### Statistical analyses

Initial analyses were descriptive. Continuous variables were expressed as means ± standard deviation (SD) or as medians with total and interquartile ranges and compared using unpaired *t*-tests or Wilcoxon rank sum tests, as appropriate. Categorical variables were expressed as proportions and compared using chi-squared tests.

Given the known decrease in 25(OH)D concentrations during the fall and winter, the season of study visit was divided into winter and non-winter, with winter broadly defined as a study date falling from November through March [23, 24]. Patient race/ethnicity was characterized as either white or non-white. Children of Hispanic ethnicity were considered non-white.

Serum 25(OH)D concentrations were used to categorize the vitamin D nutritional status of the study subjects. Previously described cutoffs for serum 25(OH)D were used. A patient was considered to have hypovitaminosis D if 25(OH)D was <30 ng/ml [16], vitamin D insufficiency if 25(OH)D was <20 ng/ml, and vitamin D deficiency if 25(OH)D was <10 ng/ml [25, 26].

### Multivariable logistic regression

To determine the characteristics associated with vitamin D insufficiency, we built an explanatory multivariable logistic regression model. Variables whose unadjusted, bivariable odds ratios (OR) had *P*<0.25 were eligible for inclusion in the multivariable model. Using a backwards deletion strategy, these variables were used to construct the adjusted multivariable model [27]. Multiplicative interaction terms were incorporated to evaluate the effects of race and season on the relationship between SSNS and vitamin D status. Overall model fit and calibration were assessed by the Hosmer-Lemeshow goodness-of-fit test [27]. Model discrimination was

assessed using the area under the receiver operator characteristics (ROC) curve. Multivariable logistic regression for hypovitaminosis D and vitamin D deficiency was not performed since few study subjects had vitamin D deficiency and nearly all subjects had hypovitaminosis D [27].

### Multiple linear regression

To determine if SSNS subject characteristics affected serum 25(OH)D concentrations, a multiple linear regression model was fit, using 25(OH)D as the dependent variable. Candidate independent variables included demographic characteristics and SSNS disease and treatment characteristics, such as duration of NS, number of relapses, time since last relapse, cumulative dose of glucocorticoids, and duration of glucocorticoid therapy.

Variables were screened using bivariable analysis. A backwards deletion strategy was used to determine the final, parsimonious regression model. Model fit was confirmed by (1) using a forward selection strategy after the bivariable analysis and (2) initially including all candidate variables and then employing a backwards deletion strategy [28]. Variables were assessed for collinearity and multiplicative interaction. Model specification, model fit, and the assumptions of the linear regression model were also assessed.

All analyses were conducted using Stata 7.0 statistical software (Stata Corporation, College Station, Tex., USA). Two-sided tests of hypotheses were used and a *P* value <0.05 was considered to be statistically significant.

## Results

### Subject and disease characteristics

The characteristics of the control and SSNS patients are presented in Table 1. The healthy controls were slightly older than the SSNS subjects. The significant male predominance in the SSNS subjects was consistent with the gender pattern of the disease [29]. The SSNS subjects had lower height and higher BMI z-scores compared with control subjects, findings consistent with long-term glucocorticoid use.

The disease and glucocorticoid treatment of the SSNS subjects are summarized in Table 2. Of the SSNS subjects, 26 (63.4%) were on glucocorticoid therapy at the time of the study visit; 14 (34.1%) of the SSNS subjects had been in remission for less than 8 weeks. There were 7 subjects that had undergone renal biopsies: 3 had mesangial proliferation, 2 had minimal change disease, and 2 had focal segmental glomerulosclerosis. Of the 41 patients, 6 patients (14.6%) had been treated with cyclo-

**Table 1** Characteristics of controls and subjects with steroid-sensitive nephrotic syndrome (SSNS) (*BMI* body mass index)

	Controls (n=94)	SSNS (n=41)	<i>P</i> value
Age (years) <sup>a</sup>	12.6±3.9	9.2±3.8	<0.0001
Range	4.0–21.4	6.2–22.0	
Sex (% male)	52.1	73.2	0.02
Race (%)			0.20
White	69.2	56.1	
Black	24.5	29.3	
Other	6.4	14.6	
Studied during winter (%)	33.0	43.9	0.23
Height z-score <sup>a</sup>	0.23±0.80	-0.12±1.11	0.07
Weight z-score <sup>a</sup>	0.19±0.85	0.90±1.38	0.03
BMI z-score <sup>a</sup>	0.07±0.90	1.24±1.09	<0.0001
Milk intake (cups/day)			
Median	2.09	2.21	0.79
Interquartile range	1.05–3.86	1.08–3.02	

<sup>a</sup> Mean±SD

sporine, while 11 patients (26.8%) had been treated with at least one course of an alkylating agent (cyclophosphamide or chlorambucil).

### Serum 25(OH)D, 1,25(OH)<sub>2</sub> D, and PTH concentrations

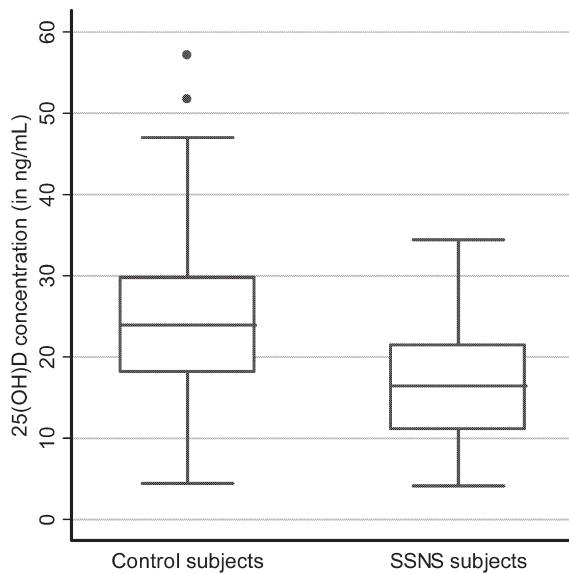
Circulating 25(OH)D concentrations were significantly lower in SSNS subjects than in control subjects. As shown in Fig. 1, SSNS subjects had a median 25(OH)D concentration of 16.4 ng/ml (mean 17.4±7.6 ng/ml), while control subjects had a median 25(OH)D concentration of 23.9 ng/ml (mean 23.9±10.3 ng/ml) (*P*=0.0002). The distributions of 25(OH)D concentrations differed significantly between the two groups, as shown in Fig. 2 (*P*=0.001). Nearly all (90.2%) of the SSNS children had hypovitaminosis D, 68.3% were vitamin D insufficient, and 19.5% were vitamin D deficient. Although most control subjects had hypovitaminosis D, only 30.8% were vitamin D insufficient and 10.6% vitamin D deficient.

Circulating 1,25(OH)<sub>2</sub> D concentrations were higher in SSNS children than in controls (Fig. 3). SSNS subjects had a median 1,25(OH)<sub>2</sub> D concentration of 47.1 pg/ml (mean 50.1±16.5 pg/ml), while control subjects had significantly lower 1,25(OH)<sub>2</sub> D concentrations, with a me-

**Table 2** Disease and treatment characteristics of SSNS subjects

	Mean±SD	Median	Range	Interquartile range
Age at diagnosis (years)	4.3±2.8	3.5	1.3–11.2	2.3–5.3
Months since last relapse	4.0±3.2	3.5	0.5–11.1	1.0–6.7
Months since last dose of glucocorticoids	0.8±2.0	0	0–8.0	0–0.1
Cumulative mg/kg glucocorticoids <sup>a</sup>	962±782	735	25–2,811	286–1,510
Cumulative mg glucocorticoids <sup>a</sup>	23,403±18,890	16,175	1,621–66,750	8,719–39,033
Number of relapses	11.9±10.5	10	0–50	4–15.5
Duration of glucocorticoid therapy (years)	4.8±3.7	4.2	0.6–17.3	1.6–6.6
Duration of NS (years)	4.9±3.7	4.2	0.6–17.5	1.6–6.6

<sup>a</sup> Between date of first dose and last dose



**Fig. 1** Box and whisker plots of 25-hydroxyvitamin D [25(OH)D] concentrations in controls and patients with steroid-sensitive nephrotic syndrome (SSNS). The *horizontal lines* within each box designate the median values, the *lower* and *upper* horizontal lines at the top and bottom of the boxes delineate the 25th and 75th percentiles, and the whiskers mark the upper and lower adjacent values. Outliers are designated by *asterisks*. The difference between the median 25(OH)D concentrations is statistically significant ( $P=0.0002$ )

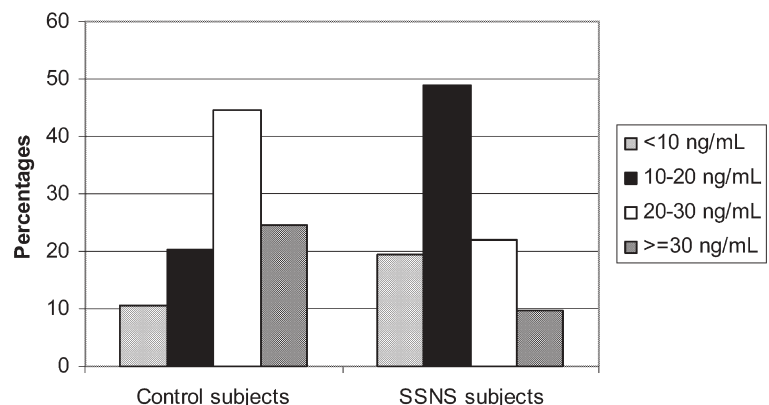
dian of 37.8 pg/ml (mean of  $41.2 \pm 14.5$  pg/ml) ( $P=0.0007$ ).

Serum PTH concentrations were available in the SSNS patients only. The mean serum intact PTH was  $22.9 \pm 10.6$  pg/ml (median 21.4 pg/ml, interquartile range 14.6–27.0 pg/ml). PTH concentrations were negatively correlated with 25(OH)D concentration ( $r=-0.30$ ,  $P=0.07$ ) and positively correlated with  $1,25(\text{OH})_2 \text{D}$  ( $r=0.3491$ ,  $P=0.03$ ).

#### Factors associated with vitamin D insufficiency

To determine factors independently associated with vitamin D insufficiency, a parsimonious multivariable logis-

**Fig. 2** Percentage of control and SSNS subjects in each category of 25(OH)D concentration. The distribution of 25(OH)D concentrations differed significantly between the control and SSNS subjects ( $P=0.001$ )

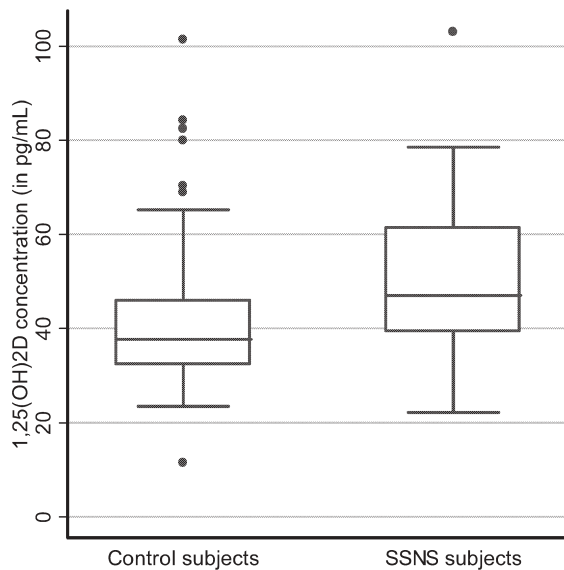


tic regression model was fit using subjects with SSNS and healthy controls (Table 3). The candidate variables were the factors listed in Table 1. The characteristics independently associated with vitamin D insufficiency were SSNS, older age, non-white race, and a study date during wintertime. Sex and anthropometric z-scores were not associated with vitamin D insufficiency in the final model. When modeled with the other variables in Table 3, increased milk intake was associated with a decreased OR of vitamin D insufficiency (OR 0.89 per cup/day milk intake, 95% confidence interval 0.76–1.05), but this association did not achieve statistical significance ( $P=0.16$ ) and was excluded from the final model. For the final model, the Hosmer-Lemeshow goodness-of-fit test statistic was 0.78. The area under the ROC curve was 0.88.

The interaction between race and season could not be tested via logistic regression, since the combination of wintertime study visit and non-white race perfectly predicted vitamin D insufficiency in both the SSNS and control subjects. All of the 20 study subjects (12 controls, 8 SSNS) who were non-white and examined during the wintertime were vitamin D insufficient. The percentage of white subjects who were vitamin D insufficient in the winter was 41.4%. This difference in proportions suggests that the impact of winter upon serum 25(OH)D may be more detrimental for non-whites than whites.

#### Factors associated with 25(OH)D concentration in subjects with SSNS

To determine whether NS disease characteristics affected 25(OH)D concentrations, a multiple linear regression model was fit, using only the SSNS patients (Table 4). Candidate variables for this model included general characteristics of all study participants (Table 1) as well as disease and treatment characteristics specific to SSNS (Table 2). After the bivariable analysis, the SSNS disease characteristics that were initially eligible for the multiple regression model were duration of NS, duration of glucocorticoid therapy, and cumulative glucocorticoid dose (unadjusted for body mass). Of note, there was no evidence of an association between serum 25(OH)D and the time interval since last relapse ( $P=0.33$ ). Age, race, height



**Fig. 3** Box and whisker plots of 1,25(OH)<sub>2</sub> D concentrations in control and SSNS patients. The *horizontal lines* within each box designate the median values, the *lower and upper horizontal lines* at the top and bottom of the boxes delineate the 25th and 75th percentiles, and the whiskers mark the upper and lower adjacent values. Outliers are designated by *asterisks*. The difference between the median 1,25(OH)<sub>2</sub> D concentrations is statistically significant ( $P=0.0007$ )

z-score, season of study visit, and milk intake were also eligible for the multiple regression model.

In the final adjusted regression model, older age, non-white race, lower milk intake, and a study visit during wintertime were each independently associated with lower 25(OH)D concentrations (Table 4). For example, 25(OH)D concentrations were 9.25 ng/ml lower, on average, in non-whites, compared with whites. After adjusting for other potential confounders, no SSNS disease

characteristics were significantly associated with circulating 25(OH)D. Duration of NS and duration of glucocorticoid therapy were strongly collinear; neither factor was significant when included individually in the model. Although its  $P$  value was  $>0.05$ , season of study visit remained in the final model, since season is a known determinant of vitamin D status, and its inclusion improved overall model fit as measured by the adjusted  $R^2$  statistic. Multiplicative interaction between race and season of study visit was evaluated but was not significant. The adjusted  $R^2$  statistic for the final model was 0.59.

## Discussion

In this cross-sectional study, children with remitted SSNS had lower serum 25(OH)D concentrations, and hence worse vitamin D nutritional status, than local healthy controls. Serum concentrations of 25(OH)D, not 1,25(OH)<sub>2</sub> D or even the 1,25(OH)<sub>2</sub> D index, are the best indicator of total body stores of vitamin D [7]. This difference in 25(OH)D concentrations was substantial and persisted after adjusting for potential confounders such as age, race, sex, anthropometric measurements, milk intake, and the season of the study visit. The odds of having vitamin D insufficiency, defined as a 25(OH)D concentration  $<20$  ng/ml, were greatly increased in children with SSNS. Older age, non-white race, and a study visit during winter were also independently associated with greater odds of vitamin D insufficiency.

To our knowledge, this study is the first to report that children with remitted NS have worse vitamin D nutritional status than healthy controls. Prior pediatric studies that documented low 25(OH)D concentrations in NS examined children with active nephrosis, either during the presenting episode or during a relapse [2, 3, 4]. Fre-

**Table 3** Multivariable logistic regression model for odds ratio (OR) of vitamin D insufficiency (CI confidence interval)

	OR	95% CI	$P$ value
SSNS subjects (vs. control subjects)	11.2	3.5–36.2	$<0.001$
Age (per year)	1.20	1.06–1.36	0.003
Non-white race (vs. white race)	12.9	4.6–36.2	$<0.001$
Study visit during winter (vs. non-winter)	6.7	2.5–18.4	$<0.001$

**Table 4** Multiple linear regression model for 25(OH)D concentration (in ng/ml) in SSNS patients only

Variable	Bivariable (unadjusted) analysis		Multivariable analysis	
	Coefficient (95% CI)	$P$ value	Coefficient (95% CI)	$P$ value
Intercept	–	–	25.70	$<0.001$
Age (per year)	–0.82 (–1.40 to –0.23)	0.008	–0.61 (–1.03 to –0.20)	0.005
Non-white race (vs. white race)	–9.75 (–13.49 to –6.01)	$<0.001$	–9.25 (–12.40 to –6.12)	$<0.001$
Study visit during winter (vs. non-winter)	–3.40 (–8.17 to 1.37)	0.157	–2.76 (–5.90 to 0.38)	0.08
Milk intake (per cup/day)	0.99 (–0.27 to 2.24)	0.120	1.07 (0.26 to 1.90)	0.01
Height z-score (per z-score SD unit)	1.42 (–0.76 to 3.59)	0.195	–	$>0.20$
Duration of NS (per 1 year)	–0.52 (–1.15 to 0.12)	0.107	–	$>0.20$
Cumulative glucocorticoid dose (per 1,000 mg)	–0.12 (–0.24 to 0.01)	0.060	–	$>0.20$
Duration of glucocorticoid therapy (per 1 year)	–0.54 (–1.17 to 0.099)	0.096	–	$>0.20$

Adjusted  $R^2=0.59$

undlich et al. [5] studied children during relapse and remission of their NS but did not include comparisons with healthy controls. Huang et al. [6] found that children with remitted NS had 25(OH)D concentrations similar to those of healthy controls but did not adjust for season, which is known to affect serum 25(OH)D [24]. In adult NS, studies of 25(OH)D have also focused upon patients with heavy or persistent proteinuria [9, 10, 11, 12] and excluded patients in remission.

In children with remitted SSNS, repeated episodes of proteinuria are the most likely cause of the low 25(OH)D concentrations. Children with SSNS usually suffer multiple relapses, with 60% experiencing five or more [14]. In this study, the median number of relapses was ten. During relapses, the response to glucocorticoids is reliable but not always instantaneous, and proteinuria may persist for weeks before resolving [17, 30]. Recurring periods of prolonged proteinuria may eventually produce deficits of circulating 25(OH)D. In this study, however, number of relapses, duration of NS, time since last relapse, and other markers of SSNS disease severity were not independently associated with the serum 25(OH)D concentration (Table 4). SSNS disease characteristics that were not measured in this study, such as cumulative duration of relapses or severity of proteinuria in each relapse, may be more closely associated with decrements in circulating 25(OH)D.

Decreases in sunlight exposure may contribute to the lower 25(OH)D concentrations in children with SSNS. Exposure to ultraviolet rays is required for dermal synthesis of vitamin D<sub>3</sub>, the precursor to 25(OH)D and 1,25(OH)<sub>2</sub> D. In SSNS children, decreased sunlight exposure may stem from decreased outdoor activity. Skin changes related to glucocorticoid use may cause SSNS children to wear additional clothing or apply topical sunscreens, two measures that prevent dermal synthesis of vitamin D<sub>3</sub> [31, 32]. Sunlight exposure is difficult to quantify, however, and was not measured in this study.

Decreased dietary intake of vitamin D is another possible cause of the lower 25(OH)D concentrations in SSNS. Children with SSNS and their families are advised to restrict sodium intake, due to edema and glucocorticoid-induced salt and water retention. In addition to being fortified with vitamin D, milk contains approximately 125 mg sodium/8 ounces. At the Children's Hospital of Philadelphia, families are counseled to limit milk intake to 1.5 cups per day due to the sodium content. In this study, the milk intake was similar between the SSNS and control groups in the month prior to the study visit. However, SSNS subjects may limit milk intake during active relapses. Finally, SSNS subjects were not grossly malnourished; in fact, 41% were obese, compared with 3% of control subjects.

Besides NS, other factors independently associated with vitamin D insufficiency were non-white race, a study visit during winter, and older age. An association of non-white race with lower circulating 25(OH)D was expected. Prior studies have found that blacks [33, 34], Asians [35], and Hispanics [36] have lower 25(OH)D concentrations

than whites. The seasonal variation of serum 25(OH)D is also well known [23] and arises from the atmospheric attenuation of ultraviolet radiation during cold-weather months, due to the increased zenith angle of the sun [24]. In contrast, the association of older age with worse vitamin D nutritional status, in both control and SSNS children, was unexpected. Older age has been associated with decreased circulating 25(OH)D, but these studies have examined the elderly [37] rather than children. Most pediatric studies of 25(OH)D have examined children within a narrow age range [23, 34, 38, 39], limiting their ability to show a relationship between 25(OH)D and age. The age-related decrease in 25(OH)D was not attributable to decreased milk intake in older children. The authors are not aware of other pediatric studies showing an age-related decrease in circulating 25(OH)D, and this finding will need to be confirmed in future studies.

The high prevalence of 25(OH)D insufficiency in the children with SSNS suggests that serum 25(OH)D may need to be routinely checked in all children with NS. In the past, altered bone and vitamin D status was thought to occur only in children with long-standing, massive proteinuria, like those with Finnish-type congenital NS or treatment-resistant NS [12, 15]. In this study, however, children with SSNS, which constitutes the vast majority of childhood idiopathic NS, had lower 25(OH)D concentrations than healthy children. Furthermore, the children with SSNS were in remission for at least 14 days prior to the date of the study visit. Because the duration of NS and the number of relapses were not significant in the multiple regression model, even children with infrequent relapses may be at risk for vitamin D insufficiency and deficiency.

The high prevalence of hypovitaminosis D and vitamin D insufficiency in children with SSNS suggests that many children with SSNS may benefit from vitamin D repletion. When serum 25(OH)D is less than 30 ng/ml, NKF guidelines recommend high-dose supplementation with ergocalciferol (vitamin D<sub>2</sub>) for at least 6 months, although these guidelines are intended for persons with GFR  $\leq$  60 ml/min and elevated PTH [16]. In this study, nearly all (90.2%) of the children with SSNS meet this cutoff [25(OH)D < 30 ng/ml] for high-dose vitamin D therapy. It is unclear, however, whether the NKF guidelines are applicable to children with SSNS with decreased 25(OH)D but normal GFR and PTH.

Although they had higher 25(OH)D concentrations than the SSNS children, the control children also had a high prevalence of hypovitaminosis D. Admittedly, there are no uniform definitions of what 25(OH)D concentrations are low, suboptimal, or normal [40]. Since population-based reference ranges vary with diet, geography, and sunlight exposure, health-based reference limits may be more appropriate. One approach defines a "low" 25(OH)D concentration (hypovitaminosis D) as the concentration of 25(OH)D above which vitamin D supplementation no longer decreases PTH [26]. Vitamin D insufficiency refers to 25(OH)D concentrations low enough to eventually cause anatomical, physiological, or bio-

chemical abnormalities, such as rickets. Vitamin D deficiency describes even lower 25(OH)D concentrations and the definite existence of such abnormalities, which may reverse with vitamin D repletion [41]. The cutoff 25(OH)D concentrations for these categories are somewhat arbitrary, although the cutoffs of 30 ng/ml, 20 ng/ml, and 10 ng/ml used in this study have been previously used [16, 26]. Several recent studies have documented that ostensibly healthy children often have hypovitaminosis D or more severe states of low 25(OH)D [23, 34, 38, 39]. Most notably, a cross-sectional study of 307 healthy adolescents from the Boston area reported a 42.0% prevalence of serum 25(OH)D concentrations  $\leq$ 20 ng/ml [39], which is even greater than the 30.9% prevalence in our healthy controls. The nearly universal occurrence of hypovitaminosis D in our controls suggests that measurement of 25(OH)D may be useful in otherwise healthy children, as well as in children with SSNS.

This study has several important limitations. Firstly, this study's cross-sectional design precludes definitive conclusions regarding causal inference. A prospective, longitudinal cohort that measures vitamin D metabolites before and after onset of SSNS would be the preferred study design. Secondly, this study examined SSNS patients from two medical centers in one city, so the results may not be applicable to other pediatric nephrology practices, elsewhere in the United States or abroad. However, these two centers draw upon a diverse patient population and serve as the main pediatric nephrology referral centers for a large metropolitan area. Thirdly, this study examined children whose SSNS had relapsed within the previous 12 months. These results may not apply to children with long-dormant SSNS, a population whose 25(OH)D status has not been addressed. Fourthly, diet and sunlight exposure may confound the relationship between SSNS and 25(OH)D, and this study collected only limited information regarding diet and no information regarding sunlight exposure. Although they are difficult to quantify, diet and sunlight exposure should be measured in future studies of SSNS to help determine whether low 25(OH)D concentrations in SSNS are due to proteinuria or due to decreased sunlight exposure and dietary intake.

Finally, the clinical significance of low 25(OH)D levels, in both the SSNS and control children, is uncertain. Surprisingly, SSNS patients did not demonstrate secondary hyperparathyroidism. Measurements of PTH were unavailable in the healthy controls, so we could not determine whether SSNS patients had higher PTH concentrations than control patients. The SSNS children in this study are unlikely to have abnormal bone densities, since they are a subset of a group of NS children whose dual-energy X-ray absorptiometry results were normal [42]. This present study did not measure serum markers of bone formation and resorption, such as bone-specific alkaline phosphatase and serum pyridinoline, or utilize bone biopsies. Further studies are necessary to determine whether low 25(OH)D status leads to clinically significant metabolic bone disease in SSNS. Low 25(OH)D con-

centrations have also been linked to non-skeletal consequences, such as certain cancers, cardiovascular disease, and type 1 diabetes [43]. Studies larger than the present one, with long-term follow-up, are required to determine whether hypovitaminosis D is associated with these diseases in persons with SSNS.

In summary, children with remitted SSNS have a worse vitamin D nutritional status, as measured by serum 25(OH)D concentrations, than healthy controls. This difference persists after adjusting for the confounding effects of age, race, season, and milk intake. Nearly all (90.2%) of the SSNS children had serum 25(OH)D concentrations less than 30 ng/ml, the cutoff below which the NKF recommends high-dose supplementation with vitamin D in persons with decreased GFR and increased PTH. Children with SSNS may benefit from routine measurement of 25(OH)D, which could lead to earlier detection of vitamin D insufficiency and earlier treatment of its consequences. Additional studies are needed to determine the clinical significance of suboptimal 25(OH)D concentrations in both healthy children and in children with remitted SSNS.

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