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GLUCOCORTICOID EFFECTS ON CHANGES IN BONE MINERAL DENSITY AND CORTICAL STRUCTURE IN CHILDHOOD NEPHROTIC SYNDROME

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Abstract

The impact of glucocorticoids (GC) on skeletal development has not been established. The objective of this study was to examine changes in volumetric BMD (vBMD) and cortical structure over one year in childhood nephrotic syndrome (NS) and to identify associations with concurrent GC exposure and growth. Fifty-six NS participants, ages 5–21 years, were enrolled a median of 4.3 (0.5, 8.1) years after diagnosis. Tibia peripheral quantitative CT (pQCT) scans were obtained at enrollment and 6 and 12 months later. Sex, race and age-specific Z-scores were generated for trabecular vBMD (TrabBMD-Z), cortical vBMD (CortBMD-Z), and cortical area (CortArea-Z) based on > 650 reference participants. CortArea-Z was further adjusted for tibia length-for-age Z-score. Quasi-least squares regression was used to identify determinants of changes in pQCT Z-scores. At enrollment, mean TrabBMD-Z (-0.54 ± 1.32) was significantly lower ($p=0.0001$) and CortBMD-Z (0.73 ± 1.16 , $p<0.0001$) and CortArea-Z (0.27 ± 0.91 , $p=0.03$) significantly greater in NS, vs. reference participants, as previously described. Forty-eight (86%) participants were treated with GC over the study interval (median dose 0.29 mg/kg/day). On average, TrabBMD-Z and CortBMD-Z did not change significantly over the study interval; however, CortArea-Z decreased ($p=0.003$). Greater GC dose ($p<0.001$), lesser increases in tibia length ($p<0.001$) and lesser increases in CortArea-Z ($p=0.003$) were independently associated with greater increases in CortBMD-Z. Greater increases in tibia length were associated with greater declines in CortArea-Z ($p < 0.01$); this association was absent in reference participants (interaction $p<0.02$). In conclusion, GC therapy was associated with increases in CortBMD-Z, potentially related to suppressed bone formation and greater secondary mineralization. Conversely, greater growth and expansion of

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CortArea-Z (i.e. new bone formation) were associated with declines in CortBMD-Z. Greater linear growth was associated with impaired expansion of cortical area in NS. Studies are needed to determine the fracture implications of these findings.

Keywords

nephrotic syndrome; glucocorticoids; pQCT; BMD; childhood

INTRODUCTION

Skeletal development in childhood is characterized by sex-, race-, and maturation-specific increases in trabecular and cortical volumetric bone mineral density (vBMD) and cortical dimensions.⁽¹⁾ Glucocorticoids (GCs) are the cornerstone of therapy of many childhood diseases. However, GCs have deleterious skeletal effects as a consequence of decreased bone formation and variable effects on bone resorption.^(2–5) The growing skeleton may be especially susceptible to these effects, potentially resulting in impaired bone modeling and remodeling. These effects have not been addressed in longitudinal studies during growth.

Nephrotic Syndrome (NS) is the most common chronic renal disease of childhood with normal renal function. NS usually responds to high-dose GC therapy (2 mg/kg/day). However, the majority of children relapse, resulting in prolonged and repeated courses of GCs.⁽⁶⁾ During remission, the underlying disease remains quiescent without detectable alterations in cytokine levels. Therefore, we submit that NS is the best clinical model of GC induced osteoporosis in childhood, with minimal confounding effects of underlying inflammation.

We recently reported that GC therapy in childhood NS was associated with greater cortical compartment vBMD, as measured by peripheral quantitative computed tomography (pQCT), and compared to healthy reference participants.⁽⁷⁾ In addition, lower levels of bone formation and resorption biomarkers were associated with significantly greater cortical vBMD. Based on the fact that mature bone is more mineralized than newly-formed bone⁽⁸⁾ and GCs inhibit bone formation, we hypothesized that suppressed bone modeling contributed to the greater cortical vBMD Z-scores observed in NS. Importantly, these analyses were limited by the cross-sectional study design and the inability to assess the impact of bone modeling, as measured by increases in bone length and cortical dimensions.

The objectives of this prospective cohort study were to extend these observations (1) to assess changes in cortical and trabecular vBMD and cortical dimensions in children and adolescents with NS over a 12-month interval, and (2) to determine the longitudinal relations between GC exposure, growth, bone biomarkers and bone accrual.

MATERIALS AND METHODS

STUDY PARTICIPANTS

Children and adolescents with a diagnosis of NS, as defined by the International Society of Kidney Disease in Children,⁽⁹⁾ were identified through a systematic review of the medical records in the Division of Nephrology at the Children's Hospital of Philadelphia (CHOP). Inclusion criteria were ages 5 to 21 years, normal estimated glomerular filtration rate (eGFR > 90 ml/min/1.73m²) as calculated by the Schwartz formula⁽¹⁰⁾ and either a history of systemic GC therapy for NS within the prior 12 months or GC initiation at enrollment in incident cases. We identified 143 potential participants through a systematic review of the medical records in the Division of Nephrology. We were able to establish contact with 107

patients that agreed to the screening questionnaire. Of these, 29 were not eligible and 10 declined participation, leaving 68 participants. This report includes a total of 56 participants that completed a minimum of the baseline and 6-month visits. A total of 49 of these participants completed the 12-month visit. The prior cross-sectional study was limited to participants in urinary remission at the time of the study visit in order to provide unbiased estimates of body weight and muscle mass in the absence of edema.⁽⁷⁾ As the focus of this study was determinants of changes in bone, and participants in a longitudinal study would likely experience relapses over the course of the study, we did not exclude participants that were not in urinary remission. Therefore this cohort includes an additional 6 participants that were ineligible for the prior study.⁽⁷⁾ Participants were excluded if they had any other illness or were receiving any medications that potentially impact growth, nutritional status, pubertal development, or bone accrual, unrelated to the treatment of NS.

NS participants were compared to a reference population of 899 healthy participants, ages 5–21 years, from the greater Philadelphia area, as previously described.⁽⁷⁾ Reference participants were excluded if they reported chronic disease or medications known to affect pubertal development, growth, nutritional status or bone accrual. Race, sex and pubertal differences in bone outcomes were recently reported in these participants.⁽¹⁾ Of these, 240 participants were enrolled in an ancillary longitudinal study with an additional visit at 12 months, as previously described.^(11–13)

The study protocol was approved by the Institutional Review Board at CHOP. Informed consent was obtained directly from participants greater than 18 years of age, and assent with parental consent in those less than 18.

ANTHROPOMETRY, PHYSICAL MATURITY AND RACE

Height was measured using a stadiometer (Holtain, Crymych, UK) and weight with a digital scale (Scaletronix, White Plains, New York). Tibia length was measured from the distal margin of the medial malleolus to the proximal border of the medial tibia condyle. Measurements were obtained in triplicate and the mean used in the analyses. Pubertal development was assessed using a self-assessment questionnaire and classified according to Tanner stage criteria.^(14,15) Study participant's race was categorized by parents or the participant according to the National Institutes of Health categories.

NS DISEASE CHARACTERISTICS AND MEDICATIONS

Medical charts were reviewed for the date of diagnosis of NS, prior therapies and current medications. Incident NS was defined as a diagnosis within 14 days prior to the baseline visit. Participants were given a medication diary at their baseline visit to record daily adjustments in prednisone doses. These records were reviewed at their study visits and reconciled with the medical record. All intravenous methylprednisolone doses were documented and converted to prednisone equivalents. The total glucocorticoid exposure was summarized as cumulative mg, mg per kg and mg per kg per day. Participants were categorized as steroid resistant according to the International Society of Kidney Disease in Children definitions.⁽⁹⁾ Many of these participants were treated with high dose intravenous methylprednisolone. Edema was assessed at the visit and rated as none, present or gross edema.

BONE ASSESSMENT BY PQCT

Bone measures were obtained in the left tibia by pQCT using a Stratec XCT2000 device (Orthometrix, White Plains, NY) with a 12-detector unit, voxel size of 0.4mm, slice thickness 2.3 mm and scan speed 25mm/sec. Stratec software version 5.5 was used to analyze all scans. A scout view was obtained to place the reference line at the proximal

border of the distal tibial growth plate in participants with open growth plates and at the distal endplate in participants with fused growth plates. Bone measurements were obtained at the 3% metaphyseal site for trabecular vBMD (mg/cm^3) and at the 38% diaphyseal site for cortical vBMD, (mg/cm^3), periosteal circumference (mm), endosteal circumference (mm), and cross sectional area (mm^2). The 38% tibia site was used to minimize partial volume effects.⁽¹⁶⁾ Fat area (mm^2) was assessed at a site 66% proximal to the distal physis. For quality assurance, the manufacturer's hydroxyapatite phantom was scanned daily. In our laboratory, the in vivo coefficient of variation (CV) ranged from 0.5 to 1.6% for pQCT outcomes in children and adolescents.

Our prior studies of cortical dimensions included assessment of the impact of muscle area.^(7,13) However, edema results in an overestimate of muscle volume by CT.⁽¹⁷⁾ Consistent with this limitation, analyses in this cohort demonstrated that decreases in serum albumin levels were associated with increases in muscle area Z-scores [β -0.22 (95% C.I., -0.35, -0.08), $p=0.002$]. In contrast, changes in serum albumin were not associated with changes in fat area [β -0.03 (95% C.I., -0.10, 0.04), $p=0.37$]. Therefore, the models assessing muscle outcomes should be interpreted with caution.

LABORATORY STUDIES

Non-fasting blood and urine specimens were collected at the time of the study visits. Serum creatinine (mg/dl) was measured by spectrophotometric enzymatic assay (Vitros, Johnson & Johnson Co, Rochester, NY) with a CV of 1% to 5%. Estimated GFR (eGFR, $\text{ml}/\text{min}/1.73\text{m}^2$) was calculated based on height and serum creatinine.⁽¹⁰⁾ Serum 25 hydroxyvitamin D [25(OH)D] was quantified by radioimmunoassay with I125-labeled tracer (DiaSorin, Stillwater, MN); the interassay and intra CV ranged from 2% to 9% and 7–11% respectively.⁽¹⁸⁾ Vitamin D deficiency was defined as a 25(OH)D level $<20\text{ng}/\text{ml}$.^(19,20) Serum albumin was measured by spectrophotometric enzymatic assay (Vitros, Johnson & Johnson Co., Rochester, NY) with a CV of 1–2%. Intact PTH (iPTH) levels were measured with the Nichols chemiluminescence assay, with an interassay CV of 7–9. Hypoalbuminemia was defined as a serum albumin level $<2.5\text{ gm}/\text{dL}$.⁽²¹⁾ Serum bone-specific alkaline phosphatase (BSAP; $\mu\text{g}/\text{L}$) was measured as a marker of bone formation in the NS participants and in 509 reference participants that agreed to phlebotomy. BSAP was performed at Quest Diagnostics Laboratories (San Juan Capistrano, CA, USA) using a two-site immunoradiometric assay with an interassay CV of 8.5%. The ratio of urinary deoxypyridinoline to creatinine (DPD; $\text{nmol}/\text{mmol creatinine}$) was performed in the NS participants and 814 reference participants. Urine DPD was assayed using high-performance liquid chromatography at Quest Diagnostics Laboratories, with an interassay CV of 7.8%.

STATISTICAL ANALYSIS

All statistical analyses were performed using Stata 11.0 (Stata Corp., College Station, TX, USA). A p value < 0.05 was considered to be statistically significant and two sided tests of hypotheses were used throughout. Continuous variables were expressed as means $\pm$ standard deviation (SD), or as median and interquartile range (IQR) if they were not normally distributed. Group differences were assessed using Student's t test or the Wilcoxon rank sum test, where appropriate. Changes within the NS participants were tested with the paired t -test or the Wilcoxon signed rank test. Correlations between continuous variables were assessed by Pearson product moment correlations or Spearman's rank correlations.

Age- and sex- specific height and body mass index (BMI , kg/m^2) Z-scores (SD scores) were calculated using National Center for Health Statistics 2000 Center for Disease Control growth data.⁽²²⁾ Sex-specific bone biomarkers Z-scores were generated within each Tanner stage based on reference participant data.⁽²³⁾ The pQCT outcomes were converted to Z-

scores using the LMS method, as previously described.^(7,11,12) This method accounts for the nonlinearity, heteroscedasticity and skew of bone data in growing children.⁽²⁴⁾ All of the pQCT Z-scores were sex- and race-specific (black versus all others) and were generated using the LMS Chartmaker Program version 2.3 in the healthy reference population.⁽²⁵⁾ The pQCT density outcomes (trabecular and cortical vBMD) were assessed relative to age. The pQCT cortical geometry outcomes were highly correlated with tibia length (all $p < 0.0001$); therefore, the Z-scores for these parameters were generated relative to age and further adjusted for tibia length for age-Z-score, according to the method of Zemel, et al.⁽²⁶⁾

Changes over each of the two 6-month intervals were examined using the multivariable quasi-least squares (QLS) regression using the `xtqls` function in Stata.^(27,28) QLS models allow for a variable number of measurements per participant. The QLS models included changes within each 6-month interval as the outcome. The following covariates were tested in all models: Z-score at the start of each interval, age, sex, race and visit. Models for changes in bone outcomes evaluated associations with concurrent tibia growth (mm), GC interval exposure both as a categorical (yes/no) and continuous (mg/kg/day) variable, interval and changes in bone biomarker Z-scores, interval and changes in 25(OH)D levels, and disease duration. Although calcineurin inhibitors may cause bone loss⁽²⁹⁾, we were unable to examine the effects of these medications on bone outcomes due to infrequent use over the study interval. The models for changes in cortical vBMD tested for an association with changes in cortical area Z-scores in addition to tibia growth in order to better understand the effects of new bone formation through linear growth and modeling of cortical dimensions. The models for changes in fat area tested GC interval exposure and tibia growth.

Multivariable regression analyses were used to examine the associations between changes in cortical dimension Z-scores and tibia growth in the NS participants and the subset of reference participants with longitudinal data. The inclusion of longitudinal controls allowed us to assess if the relations between changes in these parameters were different in NS, compared with reference participants.

RESULTS

PARTICIPANT AND DISEASE CHARACTERISTICS

Table 1 summarizes participant characteristics. The predominance of Caucasian race and male sex among NS participants was consistent with the demographics of childhood NS.⁽³⁰⁾ Eight (14%) participants were enrolled within two weeks of NS diagnosis, and the remainder a mean of 5.7 ± 4.2 years after diagnosis. Among the 15 prevalent participants that were not receiving GC therapy at the time of the baseline visit, the mean interval since last GC dose was 4.0 ± 3.6 months. Overall, 21 participants had a history of a renal biopsy demonstrating minimal change NS in ten, focal segmental glomerulosclerosis in five, mesangial proliferation in five, and membranous nephropathy in one. Edema results in an overestimate of body weight and BMI during relapses. The mean BMI-Z score was 0.87 ± 1.02 within prevalent participants in urinary remission.

CLINICAL COURSE

Twelve NS participants were steroid resistant and the remainder were steroid sensitive over the study interval. Within our study cohort, the proportions with steroid sensitive vs. steroid responsive nephrotic syndrome did not differ according to race. A summary of interval GC dose in prednisone equivalents is provided in Table 2; the cumulative glucocorticoid exposure was lower during the second 6-month interval, compared with the first 6-month interval ($p = 0.004$). Steroid resistant participants received a significantly greater GC doses

compared to steroid sensitive participants (median dose 0.70 vs. 0.22 mg/kg/day; $p = 0.005$) over the first 6 months. Median doses did not differ significantly over the subsequent interval at 0.16 and 0.04 mg/kg/day, respectively.

Over the first 6-month interval, 10 participants received solumedrol and prednisone, one participant received solumedrol alone and 35 received prednisone alone. During the second 6-month interval, 5 participants received both solumedrol and prednisone, and 28 prednisone alone. Thirty-four (72%) participants had a history of other immunosuppressant therapy prior to enrollment (calcineurin inhibitor $n = 6$, mycophenolate mofetil $n = 9$, and cyclophosphamide $n = 19$) with 9 participants receiving more than one of these immunosuppressant agents. Over the study interval 9 participants received immunosuppressive therapy (calcineurin inhibitor=3, cellcept $n=3$, and cyclophosphamide $n=4$) with one participant receiving more than one agent.

One participant sustained a clinical fracture over the study interval.

LABORATORY PARAMETERS

Laboratory parameters are summarized in Table 2. Albumin levels were below 2.5 g/dL among 16 (31%) NS participants at enrollment, including the 8 incident participants. At enrollment, 11 (79%) and 17 (46%) of black and non-black participants had 25(OH)D levels < 20 ng/mL. Baseline serum albumin and 25(OH)D levels were positively correlated ($r=0.58$, $p<0.001$), as were changes in levels over the study interval ($r=0.53$, $p<0.001$). One NS participant's serum iPTH level was above normal (78 pg/mL), the remainder were within the normal range (< 66 pg/mL). Serum albumin levels increased significantly over the study interval ($p = 0.002$). Serum 25(OH)D levels increased in both non-black and black participants; however the change was only significant among non-black participants ($p < 0.001$).

At enrollment BSAP Z-scores were significantly lower among NS participants compared to reference participants ($p < 0.001$). DPD Z-scores were lower compared to reference participants, although this difference was not statistically significant ($p = 0.54$). BSAP and DPD Z-scores increased over the study interval; however, the increases were not statistically significant. The increases in BSAP Z-scores may have been explained by the lower glucocorticoid exposure in the second 6 month interval.

The QLS model for changes in BSAP Z-scores demonstrated that greater GC doses were associated with declines in BSAP Z-scores ($p = 0.05$) and greater increases in height Z-scores were associated with greater increases in BSAP Z-scores ($p = 0.04$). Neither association was significant if both covariates were in the model. The QLS model for changes in DPD Z-scores demonstrated that greater increases in height Z-scores were associated with greater increases in DPD Z-scores ($p = 0.004$); however, there was no association between GC exposure and changes in DPD Z-scores ($p = 0.99$).

PQCT AND HEIGHT Z-SCORE AT ENROLLMENT

Table 3 summarizes pQCT and height Z-scores in incident and prevalent participants separately. The pQCT and height Z-scores among incident NS participants at enrollment were not significantly different compared to reference participants. Among prevalent participants: (1) trabecular vBMD Z-scores were significantly lower (-0.68 ± 1.24 ; $p < 0.001$), (2) cortical vBMD (0.80 ± 1.20 ; $p < 0.001$), and cortical area (0.28 ± 0.90 ; $p < 0.001$) Z-scores were significantly greater, (3) periosteal (0.26 ± 1.06 , $p = 0.11$) and endosteal (0.11 ± 1.08 , $p = 0.41$) circumference Z-scores were not significantly different, (4) fat area Z-scores were significantly greater (0.80 ± 1.12 ; $p < 0.0001$), and height Z-scores were significantly lower (-0.18 ± 0.57 , $p < 0.002$), compared to the reference participants.

CHANGES IN PQCT AND HEIGHT Z-SCORES OVER THE STUDY INTERVAL

Trabecular Density—Overall, trabecular vBMD Z-scores did not change significantly over the study interval (Table 3). However, in the QLS model for changes in trabecular vBMD Z-scores, declines in BSAP ($p = 0.038$) and DPD ($p = 0.034$) Z-scores were associated with decreases in trabecular vBMD Z-scores. Changes in trabecular vBMD Z-scores were not associated with GC exposure, disease duration or 25(OH)D levels.

Cortical Density—Overall, cortical vBMD Z-scores did not change significantly over the study interval. However, the QLS model for changes in cortical vBMD Z-scores demonstrated that greater mean GC dose [β 0.76 per mg/kg/day (95% C.I. 0.54, 0.97) $p < 0.001$], lesser increases in tibia length [β -0.04 per mm (-0.06, -0.02) $p < 0.001$], and lesser increases in cortical area Z-scores [β -1.06 per SD, 95% -1.77, -0.36 $p = 0.003$] were independently associated with greater increases in cortical vBMD Z-scores. Figure 1 illustrates the positive association between GC exposure and changes in cortical vBMD Z-scores stratified by $<$ or $\geq$ the median GC dose of 0.24 mg/kg/day.

Greater increases in BSAP Z-scores ($p = 0.03$) and DPD Z-scores ($p = 0.07$) were each associated with greater decreases in cortical vBMD Z-scores adjusted for their baseline levels. The associations with biomarker Z-scores were eliminated if either tibia growth or GC exposure was included in the model. At enrollment, serum 25(OH)D levels were positively associated with cortical BMD Z-scores in a univariate analysis ($r=0.38$, $p < 0.001$). However, in longitudinal analyses 25(OH)D levels were not associated with changes in cortical BMD Z-scores, independent of albumin levels and glucocorticoid exposure.

Cortical Dimensions—Over the study interval, cortical area, endosteal and periosteal circumference Z-scores declined significantly (Table 3). In the QLS models for changes in cortical dimension Z-scores, greater increases in tibia length were associated with greater decreases in cortical area Z-scores [β -0.01 per mm (-0.20, -0.005) $p = 0.002$], periosteal circumference Z-scores [β -0.02 (-0.03, -0.12) $p < 0.001$] and endosteal circumference Z-scores [β -0.02 (-0.03, -0.01) $p < 0.001$] among NS participants. The inverse association between changes in tibia length and periosteal circumference Z-score is illustrated in Figure 2. The inverse associations between tibia growth and changes in cortical area or periosteal circumference Z-scores were not seen in the reference participants (interaction terms $p = 0.02$ for cortical area and $p = 0.002$ for periosteal circumference). Greater baseline muscle area was significantly associated with greater increases in cortical area [β 0.09 (95% C.I., 0.03 0.14) $p = 0.001$], adjusted for serum albumin levels.

None of the changes in cortical dimension Z-scores were significantly associated with GC exposure, 25(OH)D levels or disease duration.

Fat Area—Over the study interval, fat area Z-scores decreased significantly among NS participants. In the QLS model for changes, greater increases in tibia length ($p < 0.001$) were associated with greater decreases in fat area Z-scores. Changes in fat area Z-scores were not associated with disease duration or concurrent GC exposure.

As described above, BMI and pQCT muscle area Z-scores were not included in these analyses given the effects of edema to result in an overestimate of these parameters.

Height—Overall, height Z-scores did not change significantly over the study interval. However, in the QLS model for changes in height Z-scores, greater GC dose was associated with greater declines in height Z-score ($p < 0.001$).

DISCUSSION

This study is the first to examine changes in trabecular and cortical vBMD and cortical dimensions in childhood NS. These longitudinal data showed that GC exposure was associated with decreases in a biomarker of bone formation, decreases in height Z-scores, and increases in cortical vBMD Z-scores. The novel observation of an inverse association between changes in tibia length and periosteal circumference Z-scores in NS participants, and the absence of this association in reference participants, highlights the importance of longitudinal data to characterize disease and treatment effects in childhood chronic diseases. These findings expand upon our prior observation that cortical vBMD was elevated in childhood NS and the longitudinal data provide insight into the contributions of impaired linear growth, impaired expansion of cortical dimensions, and suppression of bone formation.⁽⁷⁾

The observation of greater cortical area, periosteal and endosteal circumference Z-scores in the prevalent NS participants at enrollment, compared with reference participants, is consistent with our prior studies in NS.^{(7),(31)} Increases in fat mass are associated with increases in lean mass in healthy children and in children with GC-induced obesity.⁽³²⁾ The increased cortical dimension Z-scores in NS participants in our prior studies were attributed to increased muscle area Z-scores. Similarly, these longitudinal analyses demonstrated that greater muscle area was associated with greater increases in cortical area.

Periosteal circumference and cortical area Z-scores declined significantly over the study interval despite the fact that the Z-scores were adjusted for tibia length at each visit. The inverse association between interval changes in tibia length and cortical dimensions Z-scores suggests that the periosteal circumference failed to increase to the degree expected for the concurrent increases in tibia length. This association was not observed in the reference participants. Although we were unable to demonstrate that changes in cortical dimensions were associated with GC exposure, we hypothesize that glucocorticoids suppress this bone modeling, resulting in impaired bone accrual and declines in periosteal circumference Z-scores. Impaired periosteal modeling is consistent with established GC effects to suppress bone formation.⁽⁴⁾ Overall, the patterns of steroid dosing were highly variable across and within study participants over the year. The failure to observe associations between GC exposure and cortical dimensions (and trabecular vBMD, as noted below) may be due to the imprecision of summary measures of GC exposure that don't fully capture the variability in daily, alternate day, oral and intravenous GC regimens.

In our prior cross-sectional pQCT study in prevalent NS, we hypothesized that the marked increases in cortical vBMD Z-scores were due to GC-induced suppression of cortical modeling. That is, accumulation of older cortical bone with greater material density resulted in greater cortical vBMD. The observation that greater GC exposure, lesser linear growth, and lesser expansion of cortical area were significantly and independently associated with greater increases in cortical vBMD Z-scores is consistent with this interpretation, as is the inverse association between changes in BSAP and cortical vBMD Z-scores. Studies of anabolic bone therapies also support this interpretation, where rapid accumulation of cortical bone was associated with declines in cortical vBMD: children treated with growth hormone demonstrated significant increases in height and periosteal dimensions with concurrent declines in cortical vBMD Z-scores.⁽⁸⁾ Similarly, 1–84 PTH therapy in mature rhesus monkeys resulted in increases in cortical dimensions and the formation of new osteons with lower mineralization density.^(33,34)

Studies have consistently shown that GCs resulted in sustained reductions in bone formation because of decreased osteoblast differentiation and activity and increased osteoblast and

osteocyte apoptosis.⁽⁴⁾ However, studies of GC effects on bone resorption have produced conflicting results. GCs promote osteoclastogenesis and inhibit osteoclast apoptosis,⁽³⁾ but GCs also directly impair osteoclast adherence to bone and bone degradation, resulting in an eventual state of low bone turnover.⁽⁵⁾ In this study, we demonstrated that GC therapy was associated with declines in BSAP Z-scores, but was not associated with changes in DPD Z-scores. We also demonstrated that increases in height Z-scores were associated with significant increases in BSAP and DPD Z-scores, consistent with our studies in the reference participants.⁽²³⁾ These results illustrate the importance of considering intercurrent growth in studies of bone biomarkers in children. Importantly, the inverse associations between BSAP and cortical vBMD Z-scores persisted, despite adjustment for interval growth.

The trabecular vBMD deficits were consistent with histomorphometry studies documenting that glucocorticoids result in preferential trabecular bone loss with reductions in bone volume fraction⁽³⁵⁾ and subsequent recovery after treatment.⁽³⁶⁾ The failure to identify an association between changes in trabecular vBMD and concurrent GC exposure in NS potentially reflects the inadequate pQCT resolution to distinguish between alterations in trabecular microarchitecture and bone material density.

We were unable to detect an association between 25(OH)D levels and changes in bone outcomes despite two prior studies reporting that calciferol supplementation resulted in increases in DXA measures of areal BMD.^(37,38) However, the clinical significance of low 25(OH)D levels in patients with NS is unknown. NS is associated with urinary losses of vitamin D-binding protein and albumin, to which > 99% of circulating 25(OH)D is bound.⁽³⁹⁾ Total serum 25(OH)D levels, the conventional index of vitamin D status, may not reflect bioavailable free 25(OH)D in this setting. A recent study in healthy young adults demonstrated that bioavailable 25(OH)D levels were associated with DXA measures of areal BMD, while total 25(OH)D levels were not.⁽⁴⁰⁾ Future studies are needed to examine the relations between bioavailable 25(OH)D and trabecular and cortical outcomes in NS.

To our knowledge, prior QCT studies in adult and childhood NS are limited to three small cross-sectional studies^(41–43) and one longitudinal study.⁽⁴⁴⁾ Each of these studies reported that NS was associated with reduced trabecular BMD or bone mineral content in the spine or distal radius. Importantly, none of these studies addressed cortical vBMD or cortical structure.

Feber, et al recently examined an incident cohort of children with NS, enrolled at a mean of 19 days after diagnosis and treated with GCs according to the same regimen used in our incident participants.⁽⁴⁵⁾ Lateral thoracolumbar spine radiographs revealed a single mild vertebral deformity in 6 of 78 participants. None of the children with deformities reported back pain. Our study was limited by the lack of lateral spine radiographs, although it is notable that only one child sustained a clinical fracture during the study interval.

This study had additional limitations. We did not perform bone biopsies and were therefore not able to assess bone turnover, trabecular or cortical bone mineral density distribution, or cortical porosity. This study may have been subject to selection bias if nephrotic syndrome disease severity or response to steroids was different in the participants compared with patients that declined enrollment. Therefore, the baseline results may not be generalizable. However, the primary objective of this manuscript was to identify determinants of changes in bone outcomes. It is unlikely that the relations between exposures (such as glucocorticoid dose) and changes in bone outcomes differed in the study participants compared with those that declined participation or did not complete the follow-up study visit. The variability in participant age, growth status and disease characteristics introduced substantial heterogeneity. However the longitudinal study design allowed us to adjust for baseline

participant characteristics and bone Z-scores in order to assess the independent associations between subsequent glucocorticoid exposure and bone outcomes. Last, the modest sample size and relatively short duration of follow-up precluded any conclusions regarding fracture risk.

However, this study has multiple important strengths. It is the first longitudinal bone study using pQCT to assess cortical vBMD and cortical dimensions in childhood NS and was further strengthened by the inclusion of longitudinal data in the controls. It included a large robust reference sample allowing for adjustment of age, race, sex and tibia length in the assessment of bone outcomes and bone biomarkers.

Additional studies are necessary to determine the fracture implications of these findings and to assess the potential for recovery in the substantial proportion of NS patients that do not continue to require GC therapy in adulthood.

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Figure 1. Association between changes in Cortical BMD Z-Scores and interval GC exposure
Changes in cortical BMD Z-score are presented according to cumulative **prednisone equivalents** below vs. above the median dose of 0.24 mg/kg/day over the study interval. The mean ($\pm$ SD) change in cortical BMD Z-score was significantly greater in those treated with higher GC doses (0.62 ± 0.91 vs. -0.91 ± 1.25 ; $p = 0.002$).

Figure 2. Association between changes in periosteal circumference Z-scores and tibia growth over the study interval

Changes in periosteal circumference Z-scores were negatively correlated with tibia growth in the NS participants, $r = -0.49$, $p < 0.001$.

Table 1**Baseline Nephrotic Syndrome Characteristics**

N	56
Age (yr)	8.8 (5.1 to 21.2)
Sex, male n (%)	37 (66)
Race, n (%)	
White	34 (61)
Black	15 (27)
Other	7 (12)
Tanner 1–2, n (%)	41 (73)
Age at diagnosis (yr)	4.3 (0.5 to 15.9)
Disease duration (yr)	4.3 (0 to 16.2)

Data are presented as median (range) or n (%)

Table 2**Medication and Laboratory Parameters over the Study Interval**

	Enrollment	6 months	12 months
N	56	56	49
Glucocorticoids			
Therapy over the prior 6 months, n (%)		46 (82)	33 (67)
Prednisone Equivalent Dose, mg/kg/day *		0.38 (0.18, 0.63)	0.28 (0.12, 0.47)
Nutritional Supplements			
Calcium, n (%)	13 (23)	15 (27)	12 (35)
Dose, mg/day *	108 (81, 200)	100 (71, 200)	229 (100, 800)
Vitamin D, n (%)	22 (39)	23 (41)	17 (35)
Dose, IU/day	400 (286, 400)	400 (400, 400)	400 (400, 400)
Laboratory Parameters			
Serum 25(OH)D (ng/ml)			
Non-black race	19.4 ± 11.3	27.5 ± 14.2	27.2 ± 9.2
Black race	11.6 ± 8.2	17.7 ± 14.0	18.1 ± 13.1
Serum albumin, g/dL	3.2 (2.0, 3.9)	3.9 (3.2, 4.1)	3.9 (3.1, 4.2)
iPTH, pg/ml	23 (13, 38)	27 (19, 41)	24 (16, 32)
BSAP Z-score	-0.62 ± 1.01	-0.28 ± 0.97	-0.32 ± 1.18
	-0.80 (-1.30, 0.19)	-0.28 (-1.13, 0.37)	-0.35 (-1.07, 0.58)
DPD Z-score	-0.24 ± 1.41	0.15 ± 1.53	0.01 ± 1.78
	-0.36 (-1.19, 0.87)	0.43 (-0.69, 1.27)	0.48 (-0.42, 1.18)

Data are presented as median (interquartile range), mean ± SD, or n (%).

* Dose data exclude participants that did not receive any medication over the study interval. **All steroids were converted and are presented as prednisone equivalents.**

Table 3

pQCT and Height Z-scores at Enrollment and 12 Months

Z-scores	Incident		Prevalent		Combined Sample	
	Enrollment	12 Months	Enrollment	12 Months	12 month change	p value
Trabecular BMD	0.18 ± 1.71	0.39 ± 1.98	-0.75 ± 1.30 *	-0.80 ± 1.21 *	-0.01 ± 0.47	0.83
Cortical BMD	0.26 ± 0.59	1.56 ± 0.88	0.75 ± 1.25 *	0.29 ± 1.40	-0.20 ± 1.41	0.73
Cortical Area	0.16 ± 0.99	0.08 ± 0.88	0.23 ± 0.95	0.05 ± 0.94	-0.16 ± 0.35	0.003
Periosteal Circumference	0.13 ± 0.88	-0.01 ± 0.82	0.28 ± 1.09	0.10 ± 1.08	-0.18 ± 0.30	< 0.001
Endosteal Circumference	0.04 ± 0.65	-0.10 ± 0.65	0.23 ± 1.06	0.12 ± 1.00	-0.11 ± 0.28	0.016
Fat Area	-0.12 ± 0.94	-0.56 ± 1.23	0.78 ± 1.21 *	0.47 ± 1.28 *	-0.18 ± 0.58	0.04
Height	-0.16 ± 0.84	-0.19 ± 1.03	-0.16 ± 0.84	-0.22 ± 0.89	-0.05 ± 0.32	0.25

Results are presented as mean ± SD. These data are limited to NS participants with data at enrollment and 12 month visits

* p < 0.05 compared with reference participants