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Children with nephrotic syndrome have greater bone area but similar volumetric bone mineral density to healthy controls

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Abstract

Background—Glucocorticoid use has been associated with an increased fracture risk and reduced bone mineral density (BMD), particularly in the trabecular compartment. However the contribution of the underlying inflammatory disease process to these outcomes is poorly understood. Childhood nephrotic syndrome (NS) typically follows a relapsing-remitting course often requiring recurrent courses of glucocorticoids, but with low systemic inflammation during remission. NS therefore represents a useful clinical model to investigate the effects of glucocorticoids on BMD and bone geometry in childhood.

Methods—Children with NS were compared to age and sex matched healthy controls. Body composition and areal BMD (whole body, lumbar spine and hip) were assessed by DXA. Peripheral quantitative computed tomography (pQCT) scans were obtained at metaphyseal (4%) and diaphyseal (66%) sites of the tibia to determine volumetric BMD and bone cross-sectional geometry. Lifetime cumulative glucocorticoid exposure was calculated from medical records.

Results—29 children with NS (55% male, age 10.7±3.1years) were compared to 29 healthy controls (55% male, age 11.0±3.0years). The children with NS were of similar height SDS to controls ($p=0.28$), but were heavier ($0.65\pm1.28\text{SDS}$ vs $-0.04\pm0.89\text{SDS}$, $p=0.022$) and had greater body fat percentage SDS (0.31 ± 1.01 vs -0.52 ± 1.10 , $p=0.008$). Tibial trabecular and cortical vBMD were similar between the two groups but bone cross-sectional area (CSA) was significantly greater in children with NS at both the metaphysis ($954\pm234\text{ mm}^2$ vs $817\pm197\text{ mm}^2$, $p=0.002$) and

diaphysis ($534.9 \pm 162.7 \text{ mm}^2$ vs $463.2 \pm 155.5 \text{ mm}^2$, $p=0.014$). Endosteal and periosteal circumferences were greater in children with NS than controls (both $p<0.01$), resulting in reduced cortical thickness ($2.4 \pm 0.7 \text{ mm}$ vs $2.8 \pm 0.7 \text{ mm}$, $p=0.018$), but similar cortical CSA ($p=0.22$). The differences in cortical geometry were not statistically significant when weight was included as a confounding factor. There were no associations between cumulative steroid exposure, duration of NS or number of relapses and any bone parameter.

Conclusions—Tibial bone CSA is increased in children with NS. We speculate this is a compensatory response to increased body weight. Defects in trabecular BMD were not identified in this cohort of children with NS.

Keywords

Nephrotic Syndrome; Glucocorticoids; Bone Mineral Density; Bone Geometry; pQCT

1 Introduction

Glucocorticoids cause dose-dependent bone loss in adults which occurs rapidly on drug introduction, and is associated with increased fracture risk and secondary osteoporosis [1]. An increased fracture risk has also been reported in children requiring recurrent courses of glucocorticoids [2] and reduced bone mineral density (BMD) has been demonstrated in a number of paediatric conditions treated with glucocorticoids, including inflammatory bowel disease, systemic lupus erythematosus, inflammatory arthritides and nephrotic syndrome [3–5]. Bone strength is determined by both BMD and geometric properties of bone, yet fewer studies have attempted to elucidate the effects of glucocorticoids on bone geometry. Children treated with glucocorticoids for juvenile rheumatoid arthritis and inflammatory bowel disease have reduced bone cross-sectional area (CSA), cortical CSA and cortical thickness, as determined by peripheral quantitative computed tomography (pQCT), which result in reduced bone strength [3, 6, 7].

Importantly, the underlying inflammatory disease process may also contribute to the detrimental bone outcomes. It is recognised that pro-inflammatory cytokines may adversely affect bone formation and remodelling in children [8], and vertebral fractures have been identified at diagnosis in children with some systemic inflammatory conditions [9]. Furthermore, improvements in trabecular volumetric BMD (vBMD) following anti-inflammatory treatment for childhood Crohn's disease have been observed [3], thus highlighting the difficulties in determining the differential effects of glucocorticoids and the inflammatory processes on the growing skeleton in these clinical models.

This study aimed to examine vBMD and bone geometry using pQCT in a cohort of children with childhood nephrotic syndrome (NS). In contrast to other inflammatory conditions, NS responds rapidly to glucocorticoids with little evidence of systemic inflammation during periods of remission [10, 11]. However, the majority of children with NS will follow a relapsing-remitting course requiring multiple courses of glucocorticoids throughout childhood. Recent evidence suggests around 80% of children diagnosed with NS will have at least one relapse after initial remission, and a median of four relapses will occur [12, 13]. Studies in children using DXA have reported conflicting effects of NS treatment on bone

mineral content (BMC) [14, 15] and areal BMD (aBMD) [5, 16, 17]. In one case-controlled study using pQCT, reduced trabecular vBMD, with preservation of cortical vBMD, was reported [18]. Alterations in vBMD and geometry have also been reported in healthy overweight children [19, 20], and it is recognised that children with NS are often heavier than healthy controls. We therefore undertook this study to investigate vBMD and bone geometry in relation to body composition, disease characteristics and glucocorticoid exposure in children with NS and healthy controls.

2 Methods

2.1 Study Subjects

Children with NS were recruited from the regional tertiary nephrology clinic at University Hospitals Southampton NHS Foundation Trust, UK. Exclusion criteria included age ≥ 5 years at time of recruitment and other significant medical co-morbidity. Steroid exposure and disease characteristics were determined from medical and parent held records. A healthy control group who had never received steroid treatment and had no other chronic medical conditions was recruited by asking subjects to invite a friend of similar age to participate. Fracture history was determined in both groups by direct questioning. All fracture types (appendicular and axial) were considered.

The study was approved by the Southampton Research Ethics Committee and written informed consent was obtained from all participants and/or their parent or guardian.

2.2 Anthropometric assessment

Height was measured using a wall mounted stadiometer (Marsden HM-200) and weight to the nearest 0.1kg using electronic scales (Marsden MPPS-250). Standard deviation scores (SDS) were calculated for height, weight and body mass index (BMI) from the 1990 British reference data [21, 22]. Pubertal status was assessed by participant self-assessment using standard photographs and a Prader orchidometer, and classified according to the method of Tanner.

2.3 Dual-energy Xray Absorptiometry Scans (DXA)

Measures of BMC, aBMD and bone area were obtained using a Hologic Discovery W Dual-energy X-ray absorptiometer (Hologic, Inc., Bedford, MA, USA) with fan beam technology (software version 12.5) for whole body (WB), left hip and lumbar spine (L1-L4) (LS). aBMD SDS were provided for age and gender from the manufacturer's software. Two methods were used to minimize the effect of body size on areal BMD. Firstly, height specific SDS were calculated using published North American reference data as a method of adjusting for body size [23], and secondly, bone mineral apparent density (BMAD), a validated transformation to calculate a volumetric density from DXA data, was used. This uses the assumption that the measured site is a cylinder with a volume proportional to the second power of the projected anteroposterior area obtained from DXA measurement of areal BMD. SDS for participant age were calculated using published British reference data [24]. Body composition (fat mass and lean mass) was also determined from the whole body DXA scan. Fat-free mass (FFM) was calculated as lean mass + BMC, and fat-free mass

index (FFMI) as $\text{FFM}/\text{Height}^2$. Fat percentage and FFMI SDS for age were calculated using North American reference data for children over 8 years of age [25]. The local experimental coefficient of variation for the DXA instrument using a spine phantom was 0.68%.

2.4 Peripheral Quantitative Computed Tomography (pQCT)

pQCT scanning was performed using a Stratec XCT-2000 scanner (Stratec Inc. Pforzheim, Germany). The non-dominant leg was scanned at two sites; a metaphyseal site (largely trabecular bone) and a diaphyseal site (largely cortical bone) which corresponded to 4% and 66% of the distance from the medial malleolus to the tibial tuberosity, respectively. At each site a single 2mm thick tomographic slice was sampled at a voxel size of 0.5mm.

At the metaphyseal site, BMC, total vBMD (the mean mineral density of the total cross-section), trabecular vBMD and total bone cross-sectional area (CSA) were calculated using the manufacturer's software version 5.4. At the diaphyseal site, BMC, total vBMD, cortical vBMD, total bone cross-sectional area (CSA), cortical CSA and muscle CSA were obtained. Periosteal and endosteal circumferences and cortical thickness were calculated using the circular ring model in which bone was assumed to be a cylinder [26]. The BMC:muscle ratio was calculated as an indicator of bone mineralization relative to muscle strength [27].

Bone strength measurements were derived from the pQCT data. Torsional resistance was estimated by strength strain index (SSI) measured at the diaphyseal site [28], and bone strength index (BSI), an indicator of ability to withstand compressive forces, at the metaphyseal site [29].

The coefficient of variation for this pQCT instrument has previously been demonstrated to range from 0.88% (tibial total metaphyseal density) to 8.8% (total radial diaphyseal area), but typically 1-3% [30].

2.5 Biochemical analysis

Blood and urine samples were obtained and analysed for serum calcium, albumin and alkaline phosphatase (ALP) and urinary protein:creatinine ratio by standard laboratory analysis (Beckman UniCel DxS Synchron, Beckman Coulter (UK) Ltd, High Wycombe, UK). Serum total 25-hydroxy-vitamin D [25(OH)D] was determined using liquid chromatography mass spectrometry (Waters Acquity UPLC, Waters Cooperations, Milford, MA, USA) with 26,27-hexadeuterium-25-OH-Vitamin D3 as an internal standard. Serum parathyroid hormone (PTH) was measured using a two-site immunoenzymatic assay (Beckman Unicel DXi 800, Beckman Coulter (UK) Ltd, High Wycombe, UK).

2.6 Statistical analysis

Data was analysed using the Statistical Package for Social Sciences (SPSS) v19. Data are presented as mean $\pm$ SD unless otherwise stated. Data were checked for normality, and where necessary log-transformed. The independent t-test and Mann-Whitney U test were used to determine statistical significance between groups for normal and non-normally distributed samples, respectively. Linear regression was subsequently used to adjust for age,

gender, pubertal status and height. Correlations were assessed using Pearson coefficient and Spearman's rank. $p < 0.05$ was considered significant.

3 Results

3.1 Study Participants

Forty-five children of appropriate age were identified, of which 38 were approached with regards to study participation. Seven declined to participate in the study. Thirty-four (76%) children with NS agreed to participate in the study. Twenty-nine children (55% male, mean age 10.7 ± 3.1 years, range 6.1–17.2 years) who had complete pQCT data at both the tibial metaphysis and diaphysis were included in the analysis. Of the 5 children not included in the final analysis, one did not have pQCT scans due to scanner malfunction and the remaining four had movement artefact on the diaphyseal scan only ($n=2$) or both the diaphyseal and metaphyseal scans ($n=2$). The data were compared to 29 healthy controls of similar age and gender distribution (55% male, mean age 11.0 ± 3.0 years, range 5.7–17.7 years). Pubertal staging and ethnicity were similar between the two groups (Table 1).

The median age at diagnosis of NS was 4.7 years (range 0.7–14.3 years) and the children had been diagnosed at a median of 3.3 years (range 0.3–16.5 years) before study participation. 79% had steroid sensitive NS, of whom 52% were steroid dependent. 21% had steroid resistant NS. Only one of the children with NS had a urinary protein:creatinine ratio of >300 , and no child had clinically detectable oedema. Lifetime history of fracture was similar in the children with NS to the healthy controls (21 vs 17%, $p=0.74$).

3.2 Steroid administration

Twelve children (41%) were receiving steroid therapy at the time of study participation at a median daily prednisolone dose of 4.8 mg/m^2 (range $1.3\text{--}17.3 \text{ mg/m}^2$); for the remaining children, the median time interval since their last steroid dose was 186 days (range 2 days–3.8 years). The lifetime prednisolone exposure of the children with NS was median 9.4 g/m^2 (range $2.8\text{--}55.1 \text{ g/m}^2$).

3.3 Laboratory studies

Twenty-three children with NS and 12 controls consented to venesection. The proportion of children with $25(\text{OH})\text{D} < 50 \text{ nmol/l}$ (39% vs 33%, $p=0.74$) and $< 25 \text{ nmol/l}$ (22% vs 8%, $p=0.32$) were similar. There was no significant differences in serum albumin ($p=0.57$), corrected calcium ($p=0.65$) and ALP ($p=0.99$) in the two groups. No participant had a raised PTH. In the children with NS, there was a trend towards an association between serum albumin and $25(\text{OH})\text{D}$ ($r=0.43$, $p=0.055$) but not urinary PCR and serum $25(\text{OH})\text{D}$ ($r=-0.27$, $p=0.25$).

3.4 Body composition

The children with NS were of similar height SDS to the control group (0.14 ± 1.11 vs -0.19 ± 1.13 , $p=0.28$), but had significantly greater weight SDS (0.65 ± 1.28 vs -0.04 ± 0.89 , $p=0.022$) and BMI SDS (0.74 ± 1.37 vs 0.09 ± 0.88 , $p=0.038$).

Body fat percentage SDS was significantly greater in children with NS (0.31 ± 1.01 vs -0.52 ± 1.10 , $p=0.008$) but total fat mass, total lean mass, FFM and FFMI did not differ between the two groups (Table 1).

Children with NS receiving steroid therapy at the time of study participation tended to have greater weight SDS (1.18 ± 1.30 vs 0.27 ± 1.17 , $p=0.060$) and BMI SDS (1.32 ± 1.25 vs 0.33 ± 1.33 , $p=0.053$) than those not currently receiving steroids. Lifetime steroid exposure displayed no significant associations with height SDS ($r=-0.14$, $p=0.49$), weight SDS ($r=0.28$, $p=0.15$) nor BMI SDS ($r=0.32$, $p=0.11$), but was moderately associated with both fat percentage SDS ($r=0.42$, $p=0.049$) and FFMI SDS ($r=0.43$, $p=0.048$). Age at diagnosis and time since diagnosis were not associated with body composition outcomes.

3.5 DXA assessed BMD

Children with NS had greater SDS for aBMD for whole body (0.7 ± 0.8 vs 0.2 ± 0.7 , $p=0.026$) and LS (0.1 ± 0.9 vs -0.5 ± 0.7 , $p=0.006$), but not for left hip aBMD SDS (-0.2 ± 0.8 vs -0.1 ± 0.7 , $p=0.10$) nor FN (-0.5 ± 0.9 vs -0.8 ± 0.6 , $p=0.86$). Following correction for body size using BMAD, LS BMAD SDS remained greater in children with NS, but was of borderline significance (0.2 ± 0.9 vs -0.2 ± 0.8 , $p=0.048$). No difference was observed in FN BMAD SDS (-0.4 ± 0.9 vs -0.3 ± 1.1 , $p=0.58$). However, using height specific SDS, there were no significant differences in children with NS compared to healthy controls for whole body, lumbar spine or left hip (all $p>0.05$).

3.6 pQCT assessment

3.6.1 Tibia metaphyseal geometry and BMD—The children with NS had similar total and trabecular vBMD to healthy controls (Table 2), but had greater tibial metaphyseal cross sectional area ($954 \pm 234 \text{ mm}^2$ vs $817 \pm 197 \text{ mm}^2$, $p=0.01$), which persisted after adjustment for age, gender, ethnicity, pubertal status and height ($p=0.002$).

There were no associations between metaphyseal bone parameters and current steroid treatment, lifetime steroid exposure or time since diagnosis (data not shown). In children with NS, height ($r=0.83$, $p<0.001$), weight ($r=0.80$, $p<0.001$) and lean mass were all strongly associated with metaphyseal bone area; BMI ($r=0.51$, $p=0.064$) and FFMI ($r=0.49$, $p=0.007$) were more moderately associated. Similar associations were identified in the healthy controls (height $r=0.59$, $p=0.001$; weight $r=0.49$, $p=0.009$, lean mass $r=0.51$, $p=0.005$).

3.6.2 Tibia diaphyseal geometry and BMD—Total vBMD was lower in children with NS ($504 \pm 86 \text{ mg/cm}^3$ vs $552 \pm 86 \text{ mg/cm}^3$, adjusted $p=0.045$), but cortical vBMD was similar to healthy controls ($1026 \pm 57 \text{ mg/cm}^3$ vs $1016 \pm 69 \text{ mg/cm}^3$, adjusted $p=0.61$).

There were significant differences in bone geometry measured at the tibial diaphysis, such that children with NS had greater periosteal and endosteal circumferences than healthy controls, resulting in a reduced cortical thickness but similar cortical CSA (Table 3). Cortical geometry was not associated with lifetime steroid exposure or NS duration. However, weight was strongly associated with diaphyseal CSA in both children with NS ($r=0.84$, $p<0.001$) and healthy controls ($r=0.82$, $p<0.001$). When weight was included as a confounding factor

in the regression models, the previously observed differences in tibial diaphysis geometry between children with NS and healthy controls were no longer present (Table 3).

3.6.3 Bone strength indices—Neither BSI ($81.7 \pm 27.3 \text{ mg}^2/\text{mm}^4$ vs $66.8 \pm 33.0 \text{ mg}^2/\text{mm}^4$, $p=0.11$) nor pSSI (1145 mm^3 (IQR 932-1523 mm^3) vs 1097 mm^3 (IQR 754-1345 mm^3), $p=0.57$) differed significantly in children with NS relative to healthy controls. Strength indices also did not differ by current steroid treatment in children with NS, nor was lifetime steroid exposure associated with these outcomes.

4 Discussion

In this cross-sectional study of children with NS, we identified alterations in tibial geometry without differences in trabecular or cortical vBMD or bone strength in comparison to healthy controls. We did not identify any associations between tibial geometric parameters and disease characteristics or lifetime steroid exposure, but the heterogeneity of disease characteristics within the study cohort might have limited the power to detect these associations. However, the children with NS were heavier and had greater body fat percentage than the controls and strong associations were identified between tibial geometric properties and weight in both the children with NS and healthy controls. The differences in tibial diaphyseal geometry between the two groups were not statistically significant after adjustment for weight, suggesting weight gain associated with NS treatment might be implicated in the observed differences.

There are a number of limitations to this study. Firstly, the cross-sectional nature of the study did not allow for the observation of differences in bone outcomes in children during periods of relapse and remission. Additionally, the study is based on a relatively small cohort of children with NS over a wide range of ages, all pubertal stages and variable disease characteristics, including steroid exposure. In particular, less than half of the children were treated with glucocorticoids at the time of study participation, and the time since last steroid exposure in the remaining children ranged from a few days to several years. If recovery from deficits in bone mineralisation or geometry does occur in children without recent steroid exposure, this might have masked significant differences in those actively treated with glucocorticoids. Nonetheless, our patient population is representative of a group of patients managed in a tertiary nephrology clinic in the UK, and over 75% of eligible clinic patients agreed to participate, with 64% included in the final analysis. The peak age at diagnosis of NS is 1 to 4 years [31]. pQCT scans are exquisitely sensitive to movement artefact and, in our experience, pre-school children can find it difficult to remain still, therefore limiting its use in a large proportion of patients with NS. Furthermore, currently there is a lack of consensus on the pQCT scanning protocols for paediatric patients [32]. Age specific normative reference data are available for radial pQCT scans for children over 6 years of age [33], however no reference values are available for tibial pQCT data in children. We initially undertook radial pQCT scans in this study in order to determine differential effects on weight bearing and non-weight bearing bones, but due to the difficult positioning of children in the scanner, the degree of movement artefact rendered many of the scans un-interpretable, reducing the power of the study to detect differences. As such, we have decided not to present this data. Finally, nephrotic syndrome is more common in ethnic minorities [31], and

it is recognised that body composition and bone structure do differ by ethnicity [34]. We were not able to match the patient and control groups identically for ethnicity; however, we did adjust for ethnicity using linear regression. Despite these limitations, this data does provide an important contribution to the evolving understanding of long term effects of glucocorticoids on the developing skeleton.

Using DXA, we identified significantly greater BMD and BMAD for both whole body and lumbar spine. Previous studies using DXA to investigate bone mineralisation in childhood NS have reported conflicting results. Hegarty et al found that adults treated for childhood NS had reduced LS and femoral neck aBMD T-scores, but not age and gender specific z-scores, when using DXA manufacturer supplied reference data [16]. Their subjects had a mean final height significantly shorter than the general population and it was concluded that this reflected smaller, narrower bones although no measurement of bone area is presented [16]. A small study from Sweden, which included nine children with NS, found no difference in LS aBMD or BMC in comparison to healthy controls [35]. However, only four of the nine children had received steroid treatment within the year before study participation. Similarly, Leonard et al reported no significant effect on WB aBMC compared to healthy controls after adjusting for height, age, sex, pubertal status, ethnicity and BMI [14]. In contrast, Gulati et al reported that nearly a quarter of the one hundred children with idiopathic NS studied had osteoporosis, defined as a LS aBMD <-2.5 SDS [5], and Feber et al found that children within the first month of diagnosis of NS had a mean LS aBMD SDS significantly below the healthy average [36], but when a number of these children were followed over the first year following diagnosis of NS, LS aBMD did increase [37]. These studies also suggested that a greater cumulative prednisolone exposure was predictive of a lower LS aBMD [5, 36]. Furthermore, Mishra et al identified no difference in LS aBMD in a group of Indian children during treatment of their first presentation of steroid sensitive NS compared with children with relapsing NS [17]. No healthy control group was included in this study and, as all the children has been exposed to prednisolone, this finding might represent an effect on bone mineralisation in both treatment groups. No association between LS aBMD and prednisolone exposure was identified in this cohort [17]. Importantly, the limitations of DXA need to be considered in the interpretation of these studies. DXA is limited by the need to adjust a 2D image for body size, and many of these studies, including this study, have demonstrated differences in stature, weight and BMI in NS [14, 16, 36]. In contrast to previous studies, we found an increased WB and LS aBMD in children with NS. In our cohort, the children with NS tended to be taller than the healthy controls although not significantly. The greater BMD in the children with NS may reflect this size difference, as when BMAD was used the difference was of borderline statistical significance, and using height-adjusted SDS BMD was similar between the two groups. However, this difference further highlights the difficulties in interpreting aBMD measurements from DXA, as these differences were not observed using pQCT.

There are fewer studies using pQCT to investigate BMD and geometry in NS. In contrast to our findings, Wetzsteon et al reported a reduction in tibia trabecular vBMD SDS but increased cortical vBMD SDS in a cross-sectional study of 55 children with NS in Philadelphia, USA [18]. We did identify a mild reduction in total vBMD at the tibial diaphysis, but not metaphysis, in the children with NS. This was of borderline statistical

significance and not present after adjusting for weight. No differences in trabecular or cortical vBMD were found. Different pQCT scanning protocols were used in the studies by Wetzsteon et al and Tsampalieros et al to that followed in our study; thus the metaphyseal scan was obtained at 3% of the distance from the medial malleolus to the tibial tuberosity, compared to 4% in our study, and the diaphyseal scans at 38% and 66%, respectively [18, 38]. Whilst the scanning protocols might account for the different findings and highlights the need for a consistent approach to enable accurate comparison across studies, it is also possible that the different findings reflect heterogeneity of the study participants. Fewer children in our study were receiving steroids at the time of participation, and the interval since last steroid administration was greater in children not currently on steroids than in their cohort. Whilst reductions in LS aBMD early in the management of NS have been identified [36], it has recently been demonstrated that LS aBMD increases over the first year since glucocorticoid initiation for NS [37]. The pulsatile nature of glucocorticoid therapy in NS, or the use of steroid-sparing agents, might be important in allowing recovery from periods of poor mineralisation during steroid therapy, and could account for the different findings in this study compared to that of Wetzsteon et al. This would also be consistent with the finding that fracture risk returns to baseline in children one year post steroid exposure [2]. However, a longitudinal study from the same research group, in which many of the participants with NS from the original cross-sectional study underwent repeat pQCT after one year, found no significant change in trabecular vBMD SDS despite glucocorticoid exposure during the study period in many of the subjects [38]. This finding should therefore question the role of glucocorticoids in the trabecular demineralisation previously reported in NS. Trabecular mineralisation defects have been observed in children with juvenile rheumatoid arthritis [7] and during treatment for childhood acute lymphoblastic leukaemia [39] at cumulative glucocorticoid exposures lower than received by our study subjects, further highlighting the importance of the underlying disease process, effects of other treatment modalities, and the type of glucocorticoid used in the likelihood of mineralisation defects.

Leonard et al previously demonstrated in a study using DXA that children with NS had greater WB BMC than controls after adjustment for height, age, sex, pubertal stage and ethnicity, but the difference was no longer evident after inclusion of BMI SDS in the model [14]. We similarly found using pQCT that both tibial diaphyseal BMC, vBMD, CSA periosteal and endosteal circumferences were increased in our cohort of children with NS relative to healthy controls, but the findings were attenuated by the inclusion of weight in the statistical models. Similar increases in periosteal and endosteal circumference were identified in children with NS by Wetzsteon et al and Tsampalieros et al, however in neither study did these reach statistical significance in comparison to a reference group [18, 38]. Increased cortical CSA was reported by Wetzsteon et al, but similarly to our findings, not by Tsampalieros et al. However, the latter study, in longitudinal assessment over 12 months, did identify a reduction in periosteal and endosteal circumference z scores, which was not associated with glucocorticoid exposure, but was inversely associated with tibial length gain [38]. The differences between the three studies highlight the difficulties in cross-sectional evaluation of bone geometry in children who have a variable course of the same disease, particularly with regards to steroid exposure, and further longitudinal studies commencing at the start of relapse and several time points thereafter are now needed.

Increased bone CSA determined by pQCT has been reported in overweight, but otherwise healthy, children [19, 20, 40], and this alteration in bone geometry persists into adulthood [41]. The children with NS were heavier and had greater BMI. The alterations in bone geometry observed in our study are therefore consistent with adaptations to increased body weight that are seen in healthy overweight children. Muscle mass increases with adiposity in healthy children, yet neither DXA measured lean mass nor muscle CSA by pQCT were significantly greater in the children with NS compared to controls despite greater body weight. Nonetheless, the strong positive association of bone CSA with lean mass and weight was maintained in the children with NS. Deficits in bone strength were not identified in the children with NS and bone mineralisation relative to muscle size was similar to controls. However, given the increase in body weight, the bone strength might be insufficient to withstand the greater impact of a fall associated with higher body mass.

Fracture rate did not differ between the two groups, although this will be limited by the small number of children included in the study. However, to our knowledge an increased fracture rate has not specifically been reported in children with NS. The prospective STOPP study found that 8% of children with NS had asymptomatic vertebral fractures during their first course of glucocorticoids [36], and 6% had a vertebral fracture 12 months after initiation of glucocorticoids for NS [37]. The incidence was similar to children within 30 days of steroid initiation for rheumatological conditions [42]. Cumulative glucocorticoid exposure did not differ between those with and without vertebral fractures and no control group was included in either study to determine that this was not normal variation. Nonetheless, we did not undertake radiographic evaluation for asymptomatic vertebral fractures in either the children with NS nor healthy controls, and therefore it is possible that fracture history was underestimated.

Vitamin D deficiency has commonly been reported in NS [43, 44], however, in our study the prevalence in NS was similar to controls, and less than that reported in the contemporary studies in Philadelphia, USA [18, 38]. However, serum 25(OH)D was positively associated with serum albumin concentration, and therefore had more children who were in relapse at time of study participation been included the prevalence of vitamin D deficiency might have been higher. It is however reassuring that a satisfactory 25(OH)D status can be maintained during remission.

In conclusion, using pQCT we found no evidence of altered trabecular or cortical vBMD in the tibia of children with NS, but bone CSA was increased. This adaptation is similar to that observed in healthy overweight children. Our findings differed to those identified in a previous cohort of children with NS using pQCT, but due to the heterogeneity of steroid exposure associated with long-term treatment of NS, it remains difficult to fully understand the effects of glucocorticoids on bone from cross-sectional data. A longitudinal study with data acquisition at the start of relapse and frequent intervals thereafter is now required to more fully observe the impact of glucocorticoids and NS on bone structure.

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Reference List

- [1]. Van Staa TP, Leufkens HG, Abenham L, Zhang B, Cooper C. Use of oral corticosteroids and risk of fractures. June, 2000. *J Bone Miner Res.* 2005; 20:1487–94. [PubMed: 16149171]
- [2]. Van Staa TP, Cooper C, Leufkens HG, Bishop N. Children and the risk of fractures caused by oral corticosteroids. *J Bone Miner Res.* 2003; 18:913–8. [PubMed: 12733732]
- [3]. Dubner SE, Shults J, Baldassano RN, Zemel BS, Thayu M, Burnham JM, et al. Longitudinal assessment of bone density and structure in an incident cohort of children with Crohn's disease. *Gastroenterology.* 2009; 136:123–30. [PubMed: 19026647]
- [4]. Viswanathan A, Sylvester FA. Chronic pediatric inflammatory diseases: effects on bone. *Rev Endocr Metab Disord.* 2008; 9:107–22. [PubMed: 18165904]
- [5]. Gulati S, Godbole M, Singh U, Gulati K, Srivastava A. Are children with idiopathic nephrotic syndrome at risk for metabolic bone disease? *Am J Kidney Dis.* 2003; 41:1163–9. [PubMed: 12776267]
- [6]. Bechtold S, Alberer M, Arenz T, Putzker S, Filipiak-Pittroff B, Schwarz HP. Reduced muscle mass and bone size in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis.* 2010; 16:216–25. [PubMed: 19637389]
- [7]. Felin EM, Pahalad S, Askew EW, Moyer-Mileur LJ. Musculoskeletal abnormalities of the tibia in juvenile rheumatoid arthritis. *Arthritis Rheum.* 2007; 56:984–94. [PubMed: 17328076]
- [8]. Polzer K, Joosten L, Gasser J, Distler JH, Ruiz G, Baum W, et al. Interleukin-1 is essential for systemic inflammatory bone loss. *Ann Rheum Dis.* 2010; 69:284–90. [PubMed: 19196726]
- [9]. Huber AM, Gaboury I, Cabral DA, Lang B, Ni A, Stephure D, et al. Prevalent vertebral fractures among children initiating glucocorticoid therapy for the treatment of rheumatic disorders. *Arthritis Care Res (Hoboken).* 2010; 62:516–26. [PubMed: 20391507]
- [10]. Freundlich M, Alonzo E, Bellorin-Font E, Weisinger JR. Increased osteoblastic activity and expression of receptor activator of NF-kappaB ligand in nonuremic nephrotic syndrome. *J Am Soc Nephrol.* 2005; 16:2198–204. [PubMed: 15888564]
- [11]. Neuhaus TJ, Wadhwa M, Callard R, Barratt TM. Increased IL-2, IL-4 and interferon-gamma (IFN-gamma) in steroid-sensitive nephrotic syndrome. *Clin Exp Immunol.* 1995; 100:475–9. [PubMed: 7774059]
- [12]. Ernould S, Godron A, Nelson JR, Rigotherier C, Llanas B, Harambat J. [Idiopathic nephrotic syndrome in children: Incidence, clinical presentation, and outcome in the county of Gironde, France.]. *Arch Pediatr.* 2011
- [13]. Esfahani ST, Madani A, Asgharian F, Ataei N, Roohi A, Moghtaderi M, et al. Clinical course and outcome of children with steroid-sensitive nephrotic syndrome. *Pediatr Nephrol.* 2011; 26:1089–93. [PubMed: 21399898]
- [14]. Leonard MB, Feldman HI, Shults J, Zemel BS, Foster BJ, Stallings VA. Long-term, high-dose glucocorticoids and bone mineral content in childhood glucocorticoid-sensitive nephrotic syndrome. *N Engl J Med.* 2004; 351:868–75. [PubMed: 15329424]
- [15]. Polito C, La MA, Todisco N, Cimmaruta E, Sessa G, Pirozzi M. Bone mineral content in nephrotic children on long-term, alternate-day prednisone therapy. *Clin Pediatr (Phila).* 1995; 34:234–6. [PubMed: 7628164]
- [16]. Hegarty J, Mughal MZ, Adams J, Webb NJ. Reduced bone mineral density in adults treated with high-dose corticosteroids for childhood nephrotic syndrome. *Kidney Int.* 2005; 68:2304–9. [PubMed: 16221233]
- [17]. Mishra OP, Meena SK, Singh SK, Prasad R, Mishra RN. Bone mineral density in children with steroid-sensitive nephrotic syndrome. *Indian J Pediatr.* 2009; 76:1237–9. [PubMed: 19936659]

- [18]. Wetzsteon RJ, Shults J, Zemel BS, Gupta PU, Burnham JM, Herskovitz RM, et al. Divergent effects of glucocorticoids on cortical and trabecular compartment BMD in childhood nephrotic syndrome. *J Bone Miner Res.* 2009; 24:503–13. [PubMed: 19016583]
- [19]. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. *J Bone Miner Res.* 2008; 23:1946–53. [PubMed: 18684083]
- [20]. Ehehalt S, Binder G, Schurr N, Pfaff C, Ranke MB, Schweizer R. The functional muscle-bone unit in obese children - altered bone structure leads to normal strength strain index. *Exp Clin Endocrinol Diabetes.* 2011; 119:321–6. [PubMed: 21553367]
- [21]. Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the UK, 1990. *Arch Dis Child.* 1995; 73:25–9. [PubMed: 7639544]
- [22]. Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child.* 1995; 73:17–24. [PubMed: 7639543]
- [23]. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab.* 2010; 95:1265–73. [PubMed: 20103654]
- [24]. Ward KA, Ashby RL, Roberts SA, Adams JE, Zulf MM. UK reference data for the Hologic QDR Discovery dual-energy x ray absorptiometry scanner in healthy children and young adults aged 6–17 years. *Arch Dis Child.* 2007; 92:53–9. [PubMed: 16943261]
- [25]. Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS One.* 2009; 4:e7038. [PubMed: 19753111]
- [26]. Schoenau E, Neu CM, Rauch F, Manz F. Gender-specific pubertal changes in volumetric cortical bone mineral density at the proximal radius. *Bone.* 2002; 31:110–3. [PubMed: 12110422]
- [27]. Schoenau E, Neu CM, Beck B, Manz F, Rauch F. Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. *J Bone Miner Res.* 2002; 17:1095–101. [PubMed: 12054165]
- [28]. Ashe MC, Khan KM, Kontulainen SA, Guy P, Liu D, Beck TJ, et al. Accuracy of pQCT for evaluating the aged human radius: an ashing, histomorphometry and failure load investigation. *Osteoporos Int.* 2006; 17:1241–51. [PubMed: 16683179]
- [29]. Kontulainen SA, Johnston JD, Liu D, Leung C, Oxland TR, McKay HA. Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. *J Musculoskelet Neuronal Interact.* 2008; 8:401–9. [PubMed: 19147978]
- [30]. Oliver H, Jameson KA, Sayer AA, Cooper C, Dennison EM. Growth in early life predicts bone strength in late adulthood: the Hertfordshire Cohort Study. *Bone.* 2007; 41:400–5. [PubMed: 17599849]
- [31]. McKinney PA, Feltbower RG, Brocklebank JT, Fitzpatrick MM. Time trends and ethnic patterns of childhood nephrotic syndrome in Yorkshire, UK. *Pediatr Nephrol.* 2001; 16:1040–4. [PubMed: 11793096]
- [32]. Zemel B, Bass S, Binkley T, Ducher G, Macdonald H, McKay H, et al. Peripheral quantitative computed tomography in children and adolescents: the 2007 ISCD Pediatric Official Positions. *J Clin Densitom.* 2008; 11:59–74. [PubMed: 18442753]
- [33]. Ashby RL, Ward KA, Roberts SA, Edwards L, Mughal MZ, Adams JE. A reference database for the Stratec XCT-2000 peripheral quantitative computed tomography (pQCT) scanner in healthy children and young adults aged 6–19 years. *Osteoporos Int.* 2009; 20:1337–46. [PubMed: 19066709]
- [34]. Wetzsteon RJ, Hughes JM, Kaufman BC, Vazquez G, Stoffregen TA, Stovitz SD, et al. Ethnic differences in bone geometry and strength are apparent in childhood. *Bone.* 2009; 44:970–5. [PubMed: 19442622]
- [35]. Esbjorner E, Arvidsson B, Jones IL, Palmer M. Bone mineral content and collagen metabolites in children receiving steroid treatment for nephrotic syndrome. *Acta Paediatr.* 2001; 90:1127–30. [PubMed: 11697422]
- [36]. Feber J, Gaboury I, Ni A, Alos N, Arora S, Bell L, et al. Skeletal findings in children recently initiating glucocorticoids for the treatment of nephrotic syndrome. *Osteoporos Int.* 2012; 23:751–60. [PubMed: 21494860]

- [37]. Phan V, Blydt-Hansen T, Feber J, Alos N, Arora S, Atkinson S, et al. Skeletal findings in the first 12 months following initiation of glucocorticoid therapy for pediatric nephrotic syndrome. *Osteoporos Int*. 2013
- [38]. Tsampalieros A, Gupta P, Denburg MR, Shults J, Zemel BS, Mostoufi-Moab S, et al. Glucocorticoid effects on changes in bone mineral density and cortical structure in childhood nephrotic syndrome. *J Bone Miner Res*. 2013; 28:480–8. [PubMed: 23044926]
- [39]. Kohler JA, Moon RJ, Sands R, Doherty LJ, Taylor PA, Cooper C, et al. Selective reduction in trabecular volumetric bone mineral density during treatment for childhood acute lymphoblastic leukemia. *Bone*. 2012; 51:765–70. [PubMed: 22771958]
- [40]. Gilsanz V, Kovanlikaya A, Costin G, Roe TF, Sayre J, Kaufman F. Differential effect of gender on the sizes of the bones in the axial and appendicular skeletons. *J Clin Endocrinol Metab*. 1997; 82:1603–7. [PubMed: 9141557]
- [41]. Uusi-Rasi K, Laaksonen M, Mikkilä V, Tolonen S, Raitakari OT, Viikari J, et al. Overweight in childhood and bone density and size in adulthood. *Osteoporos Int*. 2012; 23:1453–61. [PubMed: 21850549]
- [42]. Huber AM, Gaboury I, Cabral DA, Lang B, Ni A, Stephure D, et al. Prevalent vertebral fractures among children initiating glucocorticoid therapy for the treatment of rheumatic disorders. *Arthritis Care Res (Hoboken)*. 2010; 62:516–26. [PubMed: 20391507]
- [43]. Freundlich M, Bourgoignie JJ, Zilleruelo G, Jacob AI, Canterbury JM, Strauss J. Bone modulating factors in nephrotic children with normal glomerular filtration rate. *Pediatrics*. 1985; 76:280–5. [PubMed: 3839579]
- [44]. Huang JP, Bai KM, Wang BL. Vitamin D and calcium metabolism in children with nephrotic syndrome of normal renal function. *Chin Med J (Engl)*. 1992; 105:828–32. [PubMed: 1291200]

Highlights

- Glucocorticoid use has been associated with reductions in trabecular volumetric bone mineral density (vBMD) and an increase in fracture risk.
- This study found no difference in tibial trabecular or cortical vBMD in children with nephrotic syndrome compared to healthy controls.
- Tibial cross-sectional area (CSA) was greater in children with nephrotic syndrome.
- Differences in diaphyseal CSA and cortical geometry were not significant when weight was included as a confounding factor.
- Bone geometry in childhood NS might be adapted to weight gain associated with the treatment of nephrotic syndrome.

Table 1

Anthropometric characteristics of study participants with nephrotic syndrome and healthy controls. Data displayed as mean $\pm$ SD, unless otherwise stated.

	Nephrotic syndrome	Healthy controls	P value
n (number of male)	29 (16)	29 (16)	
Age, years	10.7 $\pm$ 3.1	11.0 $\pm$ 3.0	0.71
<i>Pubertal staging (%)</i>			
Pre-pubertal (Tanner stage 1)	41	44	0.62
Peri-pubertal (Tanner stages 2–4)	52	52	
Post-pubertal (Tanner stage 5)	7	4	
<i>Ethnicity (%)</i>			
White Caucasian	72	92	0.094
Other	28	8	
Height SDS	0.14 $\pm$ 1.11	– 0.19 $\pm$ 1.13	0.28
Weight SDS	0.65 $\pm$ 1.28	– 0.04 $\pm$ 0.89	0.022
BMI SDS	0.74 $\pm$ 1.37	0.09 $\pm$ 0.88	0.038
Fat (DXA) %	31.4 $\pm$ 8.7	27.4 $\pm$ 7.4	0.063
Fat (DXA) % SDS	0.31 $\pm$ 1.01	– 0.52 $\pm$ 1.10	0.008
Fat mass (kg), median (IQR)	10.8 (6.8–16.1)	8.5 (6.1–13.4)	0.21
Lean mass (kg), median (IQR)	26.0 (19.3–30.7)	25.3 (17.6–28.4)	0.66
Fat-free mass (kg), median (IQR)	26.3 (20.2–32.1)	26.4 (18.5–29.8)	0.67
Fat-free mass index (kg/m ²), median (IQR)	12.7 (11.8–13.8)	12.4 (11.6–13.5)	0.44
Fat-free mass index SDS	– 0.19 $\pm$ 1.25	0.49 $\pm$ 0.90	0.37

Table 2

Bone geometry and volumetric mineral density of the tibial metaphysis in children with nephrotic syndrome in comparison to healthy controls (mean $\pm$ SD).

	Nephrotic syndrome	Healthy controls	P value	Adjusted p value ^a
N	29	29		
Total BMC (mg/mm)	277.1 $\pm$ 73.2	252.2 $\pm$ 56.2	0.15	0.076
Total vBMD (mg/cm ³)	291.6 $\pm$ 36.1	282.8 $\pm$ 62.3	0.52	0.45
Trabecular vBMD (mg/cm ³)	261.2 $\pm$ 45.9	260.4 $\pm$ 57.3	0.95	0.89
Total bone CSA (mm ²)	954.1 $\pm$ 234.3	816.5 $\pm$ 196.5	0.019	0.002

^a Adjusted for age, gender, ethnicity, Tanner stage, height.

Table 3

Bone geometry and volumetric mineral density of the tibial diaphysis in children with nephrotic syndrome in comparison to healthy controls (mean $\pm$ SD).

	Nephrotic syndrome	Healthy controls	P value	Adjusted p value	
				Model 1	Model 2
N	29	29			
Total BMC (mg/mm)	264.4 $\pm$ 75.8	246.4 $\pm$ 70.6	0.35	0.033	0.28
Total vBMD (mg/cm ³)	503.7 $\pm$ 85.7	551.9 $\pm$ 86.1	0.037	0.045	0.14
Total bone CSA (mm ²)	534.9 $\pm$ 162.7	463.2 $\pm$ 155.5	0.09	0.014	0.13
Cortical vBMD (mg/cm ³)	1025.6 $\pm$ 57.3	1016.0 $\pm$ 68.6	0.57	0.60	0.18
Periosteal circumference (mm)	81.1 $\pm$ 12.4	75.4 $\pm$ 12.1	0.082	0.010	0.099
Endosteal circumference (mm)	65.8 $\pm$ 13.3	57.5 $\pm$ 12.7	0.018	0.007	0.058
Cortical CSA (mm ²)	176.8 $\pm$ 57.9	187.6 $\pm$ 51.2	0.46	0.22	0.18
Cortical thickness (mm)	2.4 $\pm$ 0.7	2.8 $\pm$ 0.7	0.032	0.018	0.060
Muscle CSA (cm ²)	40.7 $\pm$ 1.4	38.1 $\pm$ 1.3	0.39	0.15	0.96
BMC:muscle CSA	6.2 $\pm$ 1.2	6.2 $\pm$ 1.3	0.92	0.34	0.26

Model 1: Adjusted for age, gender, ethnicity, tanner stage and height.

Model 2: Adjusted for age, gender, ethnicity, tanner stage, height and weight.