Cortical and Trabecular Bone Density in X-Linked Hypophosphatemic Rickets

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Context: X-linked hypophosphatemic rickets is caused by mutations in *PHEX*. Even though the disease is characterized by disordered skeletal mineralization, detailed bone densitometric studies are lacking.

Objective: The aim of the study was to assess volumetric bone mineral density (vBMD) in X-linked hypophosphatemic rickets using forearm peripheral quantitative computed tomography.

Setting: The study was conducted in the metabolic bone clinic of a pediatric orthopedic hospital.

Patients: Thirty-four patients (age, 6 to 60 years; 24 female) with *PHEX* mutations were studied, of whom 7 children (age, 6 to 11 years) were actively being treated with calcitriol and phosphate supplementation. Twenty-one patients (age, 16 to 40 years) had received the same therapy before but had discontinued the treatment; 6 patients (age, 12 to 60 years) had never received this treatment.

Main Outcome Measures: Trabecular and cortical vBMD of the radius.

Results: Trabecular vBMD was elevated (mean age-specific and sex-specific z-score: +1.0) when all patients were analyzed together, due to very high results in currently treated patients (mean z-score: +2.4) and slightly above-average mean values in the other patients. Cortical vBMD was low when the entire cohort was analyzed together (mean z-score: −3.3), but was higher in currently treated patients (mean z-score: −1.3) than in patients who had discontinued therapy (mean z-score: −3.8) or who had never been treated (mean z-score: −4.1).

Conclusions: Patients with PHEX mutations have elevated trabecular vBMD at the distal radius while receiving calcitriol and phosphate supplementation, but low cortical vBMD at the radius diaphysis. Low cortical vBMD presumably reflects the underlying mineralization defect that is not entirely corrected by current treatment approaches. **(*J Clin Endocrinol Metab* 98: E954–E961, 2013)**

Hereditary hypophosphatemic rickets is characterized by hypophosphatemia due to renal phosphate wasting, resulting in leg deformities and short stature (1, 2). Hypophosphatemic rickets is most commonly caused by mutations in the phosphate-regulating endopeptidase gene (*PHEX*), transmitted as an X-linked trait (X-linked hypophosphatemic rickets, XLH) (1, 3, 4).

The current treatment for XLH consists of oral phosphate supplementation and calcitriol and aims at maximizing growth and preventing bone deformities (1, 2). Because these main benefits are dependent on skeletal growth and the treatment schedule is quite burdensome, therapy is often discontinued once final height is achieved.

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Abbreviations: BMD, bone mineral density; LS-aBMD, lumbar spine areal bone mineral density; pQCT, peripheral quantitative computed tomography; vBMD, volumetric bone mineral density; XLH, X-linked hypophosphatemic rickets.

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Even though XLH is characterized by a generalized mineralization defect with osteomalacia on the bone histological level (5, 6), bone mineral density (BMD) results seem to be site-dependent. Areal BMD as measured by dual-energy X-ray absorptiometry is typically elevated at the lumbar spine (6–10) but low at the diaphysis of the radius (6, 7, 9, 11–13). This could reflect a differential disease effect on the axial vs the appendicular skeleton (6), or on trabecular bone (prevalent at the spine) vs cortical bone (predominant at the site of forearm densitometry). Two studies including 10 and 11 young patients, respectively, suggest that treatment with phosphate and calcitriol increases bone mineralization of the forearm shaft but does not normalize it (9, 11).

The question whether XLH affects trabecular and cortical bone differently can be investigated using peripheral quantitative computed tomography (pQCT). An early pQCT study on 9 children with hypophosphatemic rickets found normal or elevated trabecular volumetric bone mineral density (vBMD) (14). Another report mentioned extremely low cortical vBMD at the midshaft femur of 8 children with hypophosphatemic rickets (15). As cortical vBMD reflects to a large extent the average degree of mineralization of cortical bone tissue (16), it might be a useful parameter to study the effect of XLH on the skeleton and to assess changes in bone mineralization brought about by therapeutic interventions.

In the present study we therefore performed pQCT analyses at the radial metaphysis and diaphysis in 34 patients who had a diagnosis of XLH caused by *PHEX* mutations. Based on the preexisting literature, we hypothesized that trabecular vBMD was elevated in such patients but that cortical vBMD was low.

Subjects and Methods

Study population

This study included patients with hypophosphatemic rickets who were evaluated at the Shriners Hospital for Children in Montreal between 2007 and 2011. Patients younger than 6 years of age were not included, as pQCT assessments require substantial cooperation. The exclusion criterion for the densitometric studies was lower limb surgery in the past 12 months to avoid the effect of disuse on bone density results.

Of the 48 patients who fulfilled these criteria, 34 individuals (aged 6 to 60 years; 24 female, 10 male patients) were positive for disease-causing *PHEX* mutations on sequence analysis and thus had proven XLH on the molecular level. Stop mutations were found in 18 patients, frameshift mutations in 8 patients, missense mutations in 7 patients, and a splice site mutation in 1 patient. These 34 patients represent the current study population.

At the time of this study, 7 patients (age, 6.0 to 11.8 years) were actively being treated with calcitriol and phosphate supplementation and had been receiving this treatment for 1.1 to 10.5 years (mean ± SD: 6.1 ± 3.0 years). Twenty-one patients (age, 16 to 40 years) had received the same therapy before, but had discontinued the treatment when they had reached final height, 2.2 to 22.0 years (mean ± SD: 9.6 ± 6.8 years) before the study. Six patients (age, 12 to 60 years) had never received calcitriol and phosphate supplementation. The study was approved by the Institutional Review Board of McGill University. Informed consent was provided by participants or, for minors, by their parents. Assent was provided by participants aged 7 to 17 years.

Routine clinical measurements

Height was measured using a Harpenden stadiometer (Holtain, Crymych, United Kingdom). Height and weight measurements were converted to age-specific and sex-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (17). Blood and urine samples (second morning void) were obtained between 8 AM and 9 AM after an overnight fast. Serum alkaline phosphatase activity was measured using standard laboratory methods. Serum levels of intact parathyroid hormone were analyzed using a chemiluminescent immunoassay (Access Immunoassay Systems; Beckman Coulter Canada Inc, Mississauga, Canada; reference range: 1.1–6.9 pmol/L). The tubular maximum for phosphate reabsorption per liter of glomerular filtrate was determined in the fasting state using the nomogram of Walton and Bijvoet (18).

Bone densitometry

Dual-energy X-ray absorptiometry was performed in the anterior–posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 4500 device (Hologic Inc, Waltham, Massachusetts). Lumbar spine areal BMD (LS-aBMD) results were transformed to age-specific z-scores using reference data provided by the densitometer manufacturer.

Radius pQCT was performed at the nondominant forearm using Stratec XCT2000 equipment (Stratec Inc, Pforzheim, Germany) and the manufacturer's software package (version XCT 6.00B). Two sites were assessed, located at 4% and 65% of forearm length proximal to the reference line at the distal radius, as described (19, 20). These locations represent metaphysis ("4% site") and diaphysis ("65% site"), respectively. At each location, a single tomographic slice of 2.0 mm thickness was taken at a voxel size of 0.4 mm × 0.4 mm × 2 mm. At the metaphysis, the outer bone contour was detected at the default threshold of 280 mg/cm^3. The diaphysis was analyzed at a threshold of 710 mg/cm^3, using the equipment's default settings. Results of pQCT analyses were transformed to age-specific and sex-specific z-scores using our published reference data (19, 20).

Analyses of iliac bone

A bone sample was obtained from one 17-year-old patient during an orthopedic intervention, at a site 2 cm posterior to the superior anterior iliac spine. Tetracycline double labeling was performed before biopsy. Sample preparation and histomorphometric analyses were performed using previously described procedures (21). Results were compared to the average value of the age-specific reference range using reference data established in our laboratory (21). The BMD density distribution in trabecular and cortical bone from this sample was analyzed by quantitative backscattered electron imaging, as described elsewhere (22). Results were compared to the average values of a normative young reference data base established previously (23).

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Table 1. Clinical Characteristics of the Study Population

	All	Currently Treated	Previously Treated	Never Treated	*P*
N, male/female	34 (10/24)	7 (2/5)	21 (4/17)	6 (4/2)	
Age, y	23.5 (19.2 to 27.8)	8.9[a,b] (7.0 to 10.9)	26.8[c] (23.2 to 30.4)	29.0[c] (9.3 to 48.8)	.001
Height, z-score	−2.3[d] (−2.7 to −1.9)	−2.2[b] (−3.2 to −1.2)	−1.9[b] (−2.2 to −1.5)	−3.8[a,c] (−5.0 to −2.6)	<.001
Weight, z-score	0.0 (−0.4 to 0.5)	−0.3 (−0.9 to 0.4)	0.5[b] (−0.1 to 1.1)	−1.3[a] (−2.5 to 0.0)	.01
Body mass index, z-score	0.9[d] (0.6 to 1.3)	1.1 (0.3 to 1.9)	1.0 (0.5 to 1.5)	0.4 (−1.0 to 1.9)	.45
Parathyroid hormone, pmol/L	4.9 (3.5 to 6.3)	6.5 (0.0 to 13.7)	4.8 (3.8 to 5.8)	3.3 (2.0 to 4.7)	.38
Maximal tubular phosphate reabsorption/ glomerular filtration rate, mmol/L	0.57 (0.50 to 0.64)	0.51 (0.28 to 0.75)	0.60 (0.50 to 0.69)	0.54 (0.40 to 0.68)	.61
Alkaline phosphatase, U/L	201 (151 to 252)	383 (297 to 468)	134 (96 to 172)	231 (17 to 445)	<.001

Values are mean (95% confidence interval). *P* values represent the significance of the differences in mean values across the 3 subgroups, as calculated by ANOVA. The letters in superscript indicate significant differences between subgroups, as follows: [a] significantly different from result in "Previously Treated" group; [b] significantly different from result in "Never Treated" group; [c] significantly different from result in "Currently Treated" group.

The significance of the difference of mean z-scores to 0 (entire study population, column "All" only), as follows: [d] $P < .001$.

Statistical analyses

Raw results were transformed to age-specific and sex-specific z-scores from the average result in the reference population, using the published reference data cited in the description of each measurement technique. Differences between 2 groups were tested for significance using the unpaired *t* test. ANOVA was used to compare more than 2 groups. Post-hoc differences between groups were evaluated using Bonferroni's adjustment for multiple comparisons. Group differences in dichotomous variables were tested for significance using the χ^2 test. Pearson's regression coefficient was calculated for the correlation between LS-aBMD and trabecular and cortical vBMD. Stepwise multiple regression analysis was used to assess potential predictors of densitometric results. Nominal variables were coded as follows: gender: male = 1, female = 2; treatment status: never treated = 0, previously treated = 1, currently treated = 2; type of *PHEX* mutation: null mutations (stop and frameshift) = 0, other mutations = 1. All tests were 2-tailed and throughout the study a *P* value <.05 was considered significant. These calculations were performed using the SPSS Statistics software version 20.0 (SPSS Inc, Chicago, Illinois). In the iliac bone sample, estimated BMD z-scores were calculated as follows:

$$(\text{Patient\_Ca}_{\text{Mean}}/\text{Control\_Ca}_{\text{Mean}} * \text{Patient\_Md.BV/TV} - \text{Control\_Md.BV/TV})/(\text{SD of reference Md.BV/TV})$$

where $\text{Control\_Ca}_{\text{Mean}}$ and Control_Md.BV/TV are the mean or median of the reference range and Md.BV/TV is the mineralized bone volume per tissue volume.

Results

Thirty-four patients with XLH caused by *PHEX* mutations were included in this study (Table 1). LS-aBMD of the entire population was elevated (Table 2; Figure 1). Four patients had parathyroid hormone levels above the

Table 2. Densitometric Results Expressed as Age-specific and Sex-specific z-scores

	All	Currently Treated	Previously Treated	Never Treated	*P*
Lumbar spine dual-energy X-ray absorptiometry					
LS-aBMD	1.5 (0.8 to 2.1)	1.3 (0.2 to 2.5)	1.8 (0.7 to 2.8)	0.6 (−0.3 to 1.5)	.43
pQCT at radial metaphysis ("4% site")					
Total bone mineral content	0.3 (−0.3 to 0.8)	1.2 (−0.2 to 2.7)	0.2 (−0.5 to 1.0)	−0.6 (−1.6 to 0.4)	.10
Total cross-sectional area	0.6[d] (0.0 to 1.1)	−0.3 (−1.9 to 1.2)	0.8 (0.1 to 1.4)	1.0 (−0.8 to 2.9)	.20
Total vBMD	−0.2 (−0.9 to 0.5)	2.1[a,b] (1.1 to 3.0)	−0.6[c] (−1.4 to 0.2)	−1.6[c] (−3.4 to 0.1)	<.001
Trabecular vBMD	1.0[e] (0.4 to 1.5)	2.4[a,b] (1.5 to 3.4)	0.8[c] (0.1 to 1.4)	0.1[c] (−1.4 to 1.5)	.009
pQCT at radial diaphysis ("65% site")					
Total bone mineral content	−0.5[d] (−1.0 to −0.1)	−0.4 (−1.3 to 0.5)	−0.3 (−0.9 to 0.3)	−1.5 (−3.2 to 0.1)	.13
Total cross-sectional area	0.2 (−0.2 to 0.5)	−0.3 (−0.9 to 0.3)	0.4 (−0.1 to 0.9)	−0.3 (−1.8 to 1.2)	.18
Cortical cross-sectional area	−0.7[e] (−1.1 to −0.3)	−0.4 (−1.1 to 0.2)	−0.5[b] (−1.0 to 0.1)	−1.9[a] (−3.4 to −0.3)	.04
Cortical thickness	−1.0[f] (−1.4 to −0.5)	−0.3 (−0.7 to 0.1)	−0.9 (−1.5 to −0.3)	−1.9 (−3.5 to −0.4)	.07
Total vBMD	−0.8[f] (−1.1 to −0.5)	−0.2 (−0.5 to 0.2)	−0.9 (−1.4 to −0.5)	−1.2 (−2.2 to −0.2)	.08
Cortical vBMD	−3.3[f] (−4.0 to −2.6)	−1.3[a,b] (−1.9 to −0.6)	−3.8[c] (−4.7 to −2.9)	−4.1[c] (−5.5 to −2.7)	.005

Values are mean (SD). *P* values represent the significance of the differences in mean values across the 3 subgroups, as calculated by ANOVA. The letters in superscript indicate significant differences between subgroups, as follows: [a]significantly different from result in "Previously Treated" group; [b]significantly different from result in "Never Treated" group; [c]significantly different from result in "Currently Treated" group. The significance of the difference in z-scores to 0 (entire study population, column "All" only), as follows: [d]$P < .05$; [e]$P < .01$; [f]$P < .001$.

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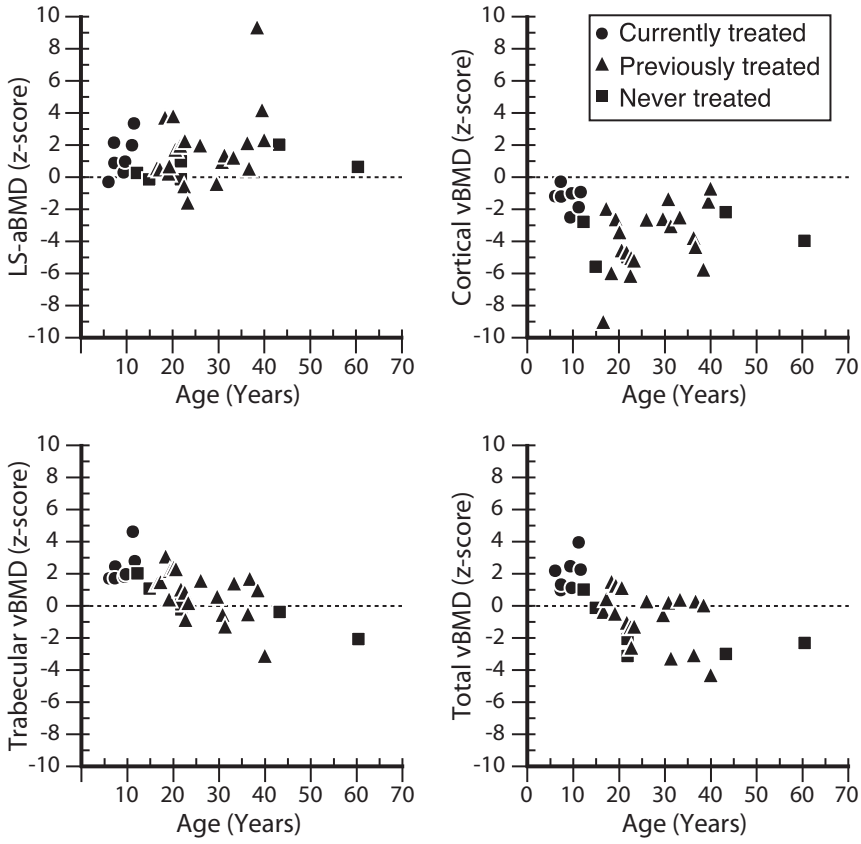

Figure 1. Individual bone density z-scores at the lumbar spine (LS-aBMD), radius diaphyseal cortex (cortical vBMD), and radius metaphysis (trabecular vBMD and total vBMD).

reference range (between 7.1 and 19.7 pmol/L). There were significant age differences between patients who were currently receiving treatment with phosphate and calcitriol (as these were children aged 6 to 11 y) and patients who had discontinued or who had never received this treatment (who were mostly aged 16 y or older). Height and weight z-scores were lowest in patients who had never received treatment, whereas LS-aBMD z-score did not vary with treatment status (Table 1; Figure 1).

Figure 2 shows examples of pQCT scans. At the radial metaphysis, total bone mineral content was normal and total bone cross-sectional area was elevated when all patients were analyzed as one group, without significant differences between subgroups (Figure 1; Table 2). Metaphyseal total vBMD in the whole patient cohort was not significantly different from the expected result in healthy subjects, but the currently treated group had high total vBMD, whereas previously treated and never-treated patients tended to have low total vBMD. Trabecular vBMD was significantly elevated when all patients were analyzed as one group, due to very high results in currently treated patients, and slightly above average mean values in the other 2 subgroups.

At the radial diaphysis, the entire patient cohort had significantly low values for total bone mineral content, cortical cross-sectional area, cortical thickness, total vBMD, and, in particular, cortical vBMD (Figure 1; Table 2). None of the 34 patients had a cortical vBMD z-score above 0. Nevertheless, cortical vBMD z-scores clearly varied with treatment status. Only 1 of the 7 patients (14%) who were currently treated with phosphate and calcitriol had a cortical vBMD z-score below −2, whereas 17 of the 21 patients (81%) who were no longer treated and all 6 patients (100%) who had never received treatment had a cortical vBMD z-score below −2.

The mean intraindividual difference between trabecular and cortical vBMD z-scores was 4.4 (95% CI: 3.5 to 5.2) and this difference did not vary with treatment status ($P = .68$). No significant association was found between LS-aBMD z-scores and either trabecular vBMD ($r = 0.11$; $P = .27$) or cortical vBMD ($r = 0.01$; $P = .49$) at the radius.

Regression analyses were performed to determine which of a set of patient characteristics (gender, age, height z-score, weight z-score, serum parathyroid hormone concentration, serum alkaline phosphatase activity, maximal tubular phosphate reabsorption/glomerular filtration rate, treatment history status, type of *PHEX* mutation) were independent predictors of densitometric results (Table 3). This revealed that weight z-score was the only determinant of LS-aBMD z-score, as well as of z-scores of total bone mineral content, total cross-sectional area, and cortical cross-sectional area at the 65% site. Age was a determinant of total and trabecular vBMD z-score at the 4% site. Treatment status was significantly associated with total vBMD at the 4% site and cortical vBMD at the 65% site. Gender, height z-score, serum parathyroid hormone levels, alkaline phosphatase activity, maximal tubular phosphate reabsorption/glomerular filtration rate, and type of *PHEX* mutation were not significantly correlated with any of the densitometric z-score results.

One 17-year-old male study participant underwent iliac bone biopsy 18 months after treatment with phosphate supplementation and calcitriol had been discontinued (Figure 3). Radius pQCT showed a trabecular vBMD of 369 mg/cm^3 (z-score: +3.1) and a cortical vBMD of 904 mg/cm^3 (z-score: −6.0). The biopsy sample contained only blurred tetracycline label, indicating a mineralization

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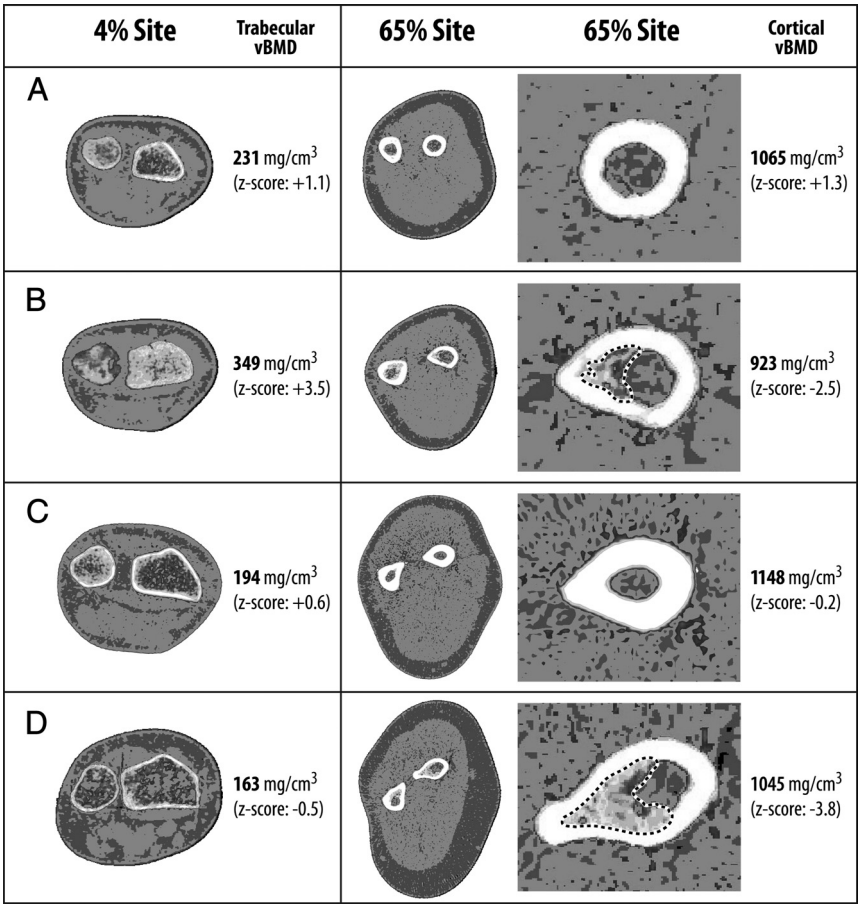

Figure 2. Forearm pQCT images at the 4% site (metaphysis) and at the 65% site (diaphysis). The panels on the right show a magnification of the radius image at the 65% site. A, Healthy male control, 10 years. B, Boy with XLH, 10 years. C, Healthy female control, 36 years. D, Female XLH patient, 37 years, mother of patient shown in (B). The encircled areas in the right panels of the XLH patients (B and D) represent regions with a mineral density between 150 and 250 mg/cm^3 (which corresponds to the density of trabecular bone). Such areas were consistently present in the diaphyseal marrow cavity of patients with XLH, but not in healthy subjects. The significance of this observation is unclear at present.

defect. The amount of unmineralized osteoid was markedly increased in both trabecular and cortical bone (Table 4). Mineralized bone volume per tissue volume was very high in trabecular bone (z-score: +4.9) but very low in cortical bone (z-score: −3.2).

Quantitative backscattered electron imaging in this sample showed that mineralized bone, both in the cortex and in the trabecular compartment, contained an abnormally large proportion of hypomineralized bone (ie, increased frequency of Ca_{Low}) (Figure 3). The mean degree of mineralization (Ca_{Mean}) was about 10% lower than the average value of the reference range. Taking into account this mineralization deficit in mineralized bone, the estimated BMD in this sample corresponds to a z-score of +3.9 for trabecular bone and −5.0 for cortical bone. These z-scores are similar to the corresponding results for the above-mentioned trabecular and cortical bone vBMD z-scores in the pQCT analysis of this patient.

Discussion

In this study we confirmed our main hypothesis that trabecular vBMD is elevated but cortical vBMD is low in patients with XLH caused by *PHEX* mutations. However, we also made the unexpected observation that trabecular vBMD results varied with age and/or treatment with phosphate supplementation and calcitriol. Children receiving this treatment had high trabecular vBMD, whereas trabecular vBMD was lower in older patients who had discontinued treatment or who had never received it. Cortical vBMD was low at all ages and treatment groups, but z-scores were

Table 3. Predictors of Densitometric Results

	Regression Equation	r^2	*P*
Lumbar spine dual-energy X-ray absorptiometry aBMD	1.21 + 0.35 × (weight, z-score)	0.13	.04
pQCT at radial metaphysis ("4% site")			
Total bone mineral content	No predictors	na	ns
Total cross-sectional area	No predictors	na	ns
Total vBMD	0.10 − 0.072 × (age, y) + 1.11 × (treatment status)	0.49	<.001
Trabecular vBMD	3.07 − 0.091 × (age, y)	0.46	<.001
pQCT at radial diaphysis ("65% site")			
Total bone mineral content	−0.50 + 0.44 × (weight, z-score)	0.20	.009
Total cross-sectional area	0.14 + 0.36 × (weight, z-score)	0.21	.008
Cortical cross-sectional area	−0.65 + 0.41 × (weight, z-score)	0.20	.01
Cortical thickness	No predictors	na	ns
Total vBMD	No predictors	na	ns
Cortical vBMD	−5.16 + 1.70 × (treatment status)	0.27	.02

Abbreviations: na, not applicable; ns, not significant. All densitometric measures are expressed as age-specific and sex-specific z-scores.

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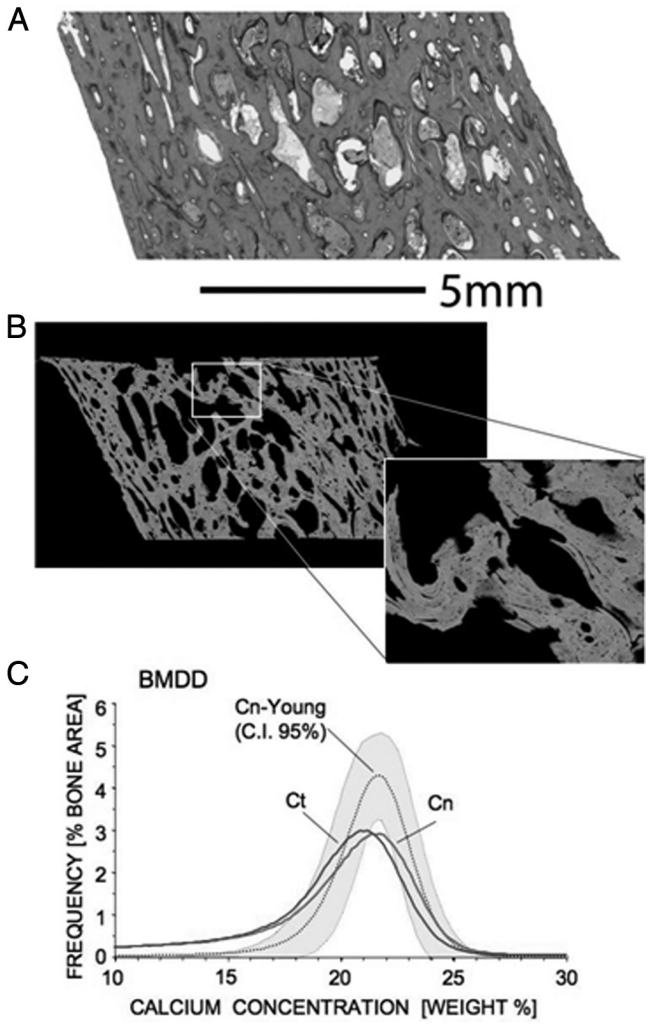

Figure 3. Iliac bone sample of a 17-year-old male patient with XLH. A, Goldner-stained section, showing a large amount of mineralized bone (light gray) and unmineralized osteoid (dark gray). B, Backscattered electron image of the same sample. Only mineralized bone is shown; areas of unmineralized bone therefore appear as porosities. C, bone mineral density distribution (BMDD) of cancellous (Cn) and cortical (Ct) bone in this sample compared to the reference distribution for children and adolescents (Cn-Young). The frequency of bone areas with low calcium concentrations (low material density) is increased in the patient sample. CI, confidence interval.

relatively higher in currently treated children than in the other 2 groups of patients.

Finding low cortical vBMD in XLH was expected, as histomorphometric studies had reported a large amount of unmineralized osteoid in cortical bone (24). This was also evident in the biopsy specimen described here. Quantitative backscattered electron imaging in one patient suggested that even the portions of the bone tissue that appear mineralized on Goldner-stained histological sections can be undermineralized. It is possible that increased cortical porosity also contributed to low cortical vBMD in our patients, as the resolution of the pQCT device used in this study is not sufficient to distinguish between the effects of low cortical mineralization and high cortical porosity (20). Cortical porosity is elevated in hyperparathyroidism, a relatively common occurrence in XLH (1), which was also evident in a few study participants. However, our results do not suggest a major effect of parathyroid hormone on cortical bone in the present study population, as multiple regression analyses did not reveal an independent association between parathyroid hormone levels and any of the densitometric parameters.

It seems that treatment with phosphate and calcitriol had some positive effect on cortical bone mineralization, as cortical vBMD z-scores were higher in children who received this treatment than in the groups who had discontinued treatment or had never received treatment. This is in accordance with histomorphometric studies that demonstrated a decrease in the amount of unmineralized osteoid when young patients with hypophosphatemic rickets started therapy with phosphate and calcitriol (24). These histomorphometric studies as well as our cortical vBMD results also demonstrate, however, that the response to treatment is incomplete and that the mineralization defect worsens after treatment discontinuation (25). It is a matter of debate whether the mineralization defect in adults should be treated with phosphate and calcitriol, but newer treatment approaches might shift the risk-benefit ratio (1).

The mineralization defect expressed by low cortical vBMD should also be operative in trabecular bone. Indeed, histomorphometric reports concur that the amount of unmineralized osteoid is markedly elevated in the trabecular bone of patients with hypophosphatemic rickets (5, 25, 26). The biopsy specimen presented here in addition suggests that in XLH mineralized bone tissue is as undermineralized in trabecular as in cortical bone. So why do patients with XLH have higher trabecular than cortical vBMD z-scores? Trabecular vBMD reflects the average amount of mineral per unit volume of the trabecular bone compartment (16). High trabecular vBMD in the presence of low average mineralization of bone tissue can therefore only occur when the amount of mineralized trabecular bone tissue is increased. This was directly observed in the iliac bone sample shown here, where mineralized bone tissue occupied 50% of the trabecular compartment, compared to a mean of 27% in the age-specific reference group (21). Similarly, histomorphometric studies in adults have shown that despite the increase in unmineralized osteoid, the amount of calcified trabecular bone volume is elevated in untreated adults with hypophosphatemic rickets (25). Such a "compensatory" mechanism is not feasible in cortical bone, because bone tissue takes up more than 90% of cortical bone volume (27), and therefore, the scope for increasing the amount of bone tissue in the cortex is very limited.

The mechanisms leading to an increased amount of trabecular bone tissue in XLH remain to be elucidated. It is

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Table 4. Histomorphometric and Quantitative Backscattered Electron Imaging Results in a 17-year-old Male Patient Compared to Age-Matched Control Values (11, 20)

	Trabecular Bone		Cortical Bone	
	Patient	Reference Data	Patient	Reference Data
Histomorphometry				
Bone volume per tissue volume, %	59.5	27.8 (4.5)	88.4	93.4 (5.0)
Mineralized bone volume per tissue volume, %	49.7	27.4 (4.6)	76.8	92.8 (5.0)
Osteoid thickness, μm	31.4	6.9 (1.2)	34.6	7.7 (3.3)
Osteoid surface per bone surface, %	84	17 (5)	80.7	22 (14)
Osteoid volume per bone volume, %	16.5	1.6 (0.7)	11.6	0.6 (0.6)
Quantitative backscattered electron imaging				
Ca_{Mean}, wt % Ca	19.1	21.0 (0.6)	18.6	20.5 (19.7; 21.0)
Ca_{Peak}, wt % Ca	21.5	21.7 (0.5)	21.0	21.1 (20.6; 21.8)
Ca_{Width}, Δwt % Ca	4.9	3.5 (3.1; 3.6)	4.7	3.8 (3.4; 4.4)
Ca_{Low}, %	23.8	6.1 (4.9; 8.0)	25.8	9.1 (6.2; 15.0)
Ca_{High}, %	1.2	0.9 (0.4; 1.5)	0.6	0.5 (0.3; 1.2)

Abbreviations: Ca_{High}, fraction of high mineralized bone (>25.30 wt %); Ca_{Low}, fraction of low mineralized bone (<17.68 wt %); Ca_{Mean}, weighted mean Ca content; Ca_{Peak}, most frequently measured Ca content; Ca_{Width}, full width at half-maximum of BMDD peak (heterogeneity of mineralization). Reference data show mean values with (SD) or median values with (25th; 75th percentiles).

possible that treatment with phosphate and calcitriol stimulates the production of trabecular bone. This hypothesis is compatible with our observation that patients who had discontinued this treatment or those who had never received it had lower trabecular vBMD z-scores than the treated group. However, the difference between trabecular and cortical vBMD z-scores was similar in all 3 treatment groups. It therefore seems that treatment with phosphate and calcitriol leads to increased mineralization of both trabecular and cortical bone and that this effect is lost once treatment is discontinued. The increased amount of trabecular bone tissue in XLH is thus probably at least partly caused by the disease process. This is also suggested by the histomorphometric observation that untreated adults with hypophosphatemic rickets have an increased trabecular bone volume (mineralized and unmineralized bone combined) (25).

In this study, we did not find a relationship between LS-aBMD and trabecular or cortical vBMD. It is possible that LS-aBMD results were influenced by paraspinal enthesopathy and spinal ligament calcification in some patients, which can develop in adults with XLH (1). However, regression analyses did not reveal an association between age and LS-aBMD z-score, suggesting that such extraosseous calcifications were not frequent in the present study population. The lack of association between LS-aBMD and trabecular and cortical vBMD might reflect the fact that LS-aBMD is influenced by a number of bone characteristics (such as bone size and geometry) that do not affect trabecular and cortical vBMD. In addition, the measurement area included in LS-aBMD scans contains both trabecular and cortical bones. As the mineral densities of these 2 bone tissues types diverge in XLH, it is expected that LS-aBMD does not correlate well with either trabecular or cortical vBMD.

This study has several obvious limitations. It is a cross-sectional study and it was therefore not possible to assess dynamic changes over time. Importantly, none of the adult study participants was taking treatment with phosphate and calcitriol, whereas most of the children were receiving this treatment. It is therefore difficult to determine whether differences between currently treated and previously treated patients represent age-dependent changes or rather reflect the waning effect of discontinued therapy in the older group. Nevertheless, this is the first detailed study on radius trabecular and cortical vBMD in XLH. It is also the first densitometric study on XLH whereby the diagnosis was confirmed by *PHEX* sequence analysis and thus the study population is homogeneous on the genetic level.

In conclusion, patients with *PHEX* mutations have elevated trabecular vBMD at the distal radius while receiving calcitriol and phosphate supplementation, but low cortical vBMD at the radius diaphysis. Low cortical vBMD presumably reflects the underlying mineralization defect that is not entirely corrected by current treatment approaches.

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