Early Skeletal and Biochemical Alterations in Pediatric Chronic Kidney Disease

Katherine Wesseling-Perry,* Renata C. Pereira,* Chi-Hong Tseng,† Robert Elashoff,‡ Joshua J. Zaritsky,* Ora Yadin,* Shobha Sahney,§ Barbara Gales,* Harald Jüppner,§ and Isidro B. Salusky*

Summary

Background and objectives The relationship between parathyroid hormone, fibroblast growth factor 23 (FGF-23), and indices of bone turnover and mineralization in children with early CKD is unknown; thus, this study characterizes the features of renal osteodystrophy and their relationship to biochemical markers of mineral metabolism.

Design, setting, participants, & measurements Fifty-two patients 2–21 years of age with predialysis CKD underwent tetracycline-labeled bone biopsy. Anthropomorphic measurements and biochemical values were obtained at the time of biopsy.

Results Serum phosphorus levels were increased in 4% of patients with stage 3 CKD and 43% of those with stage 4/5 CKD. Parathyroid hormone concentrations were elevated in 36% of patients with stage 2, 71% with stage 3, and 93% with stage 4/5 CKD, whereas FGF-23 values were elevated in 81% of all patients, regardless of CKD stage. Bone turnover was normal in all patients with stage 2, but was increased in 13% with stage 3 and 29% with stage 4/5 CKD. Defective mineralization was present in 29% of patients with stage 2, 42% with stage 3, and 79% with stage 4/5 CKD. Defective skeletal mineralization was associated with lower serum calcium levels and increased parathyroid hormone concentrations.

Conclusions Elevated circulating FGF-23 levels and defects in skeletal mineralization early in the course of CKD suggest that factors other than the traditional markers of mineral deficiency play a crucial role in the development of renal bone disease.


Introduction

Because CKD in childhood leads to growth failure, metabolic bone disease, and cardiovascular disease, severely affecting quality of life, as well as shortening lifespan (1), diagnostic and therapeutic approaches to CKD-mineral bone disease (CKD-MBD) ideally must emphasize primary prevention, early detection, and management to ameliorate CKD progression and to prevent its complications. Serum parathyroid hormone (PTH) levels are used as a biomarker of bone turnover; however, current treatment recommendations are opinion based and controversial in pediatric patients with predialysis CKD (2,3). Some data suggest that optimal growth is associated with serum PTH levels within the normal range (4), whereas other studies have demonstrated the greatest growth velocity at higher PTH levels (5). Although PTH levels are currently used to guide therapy with active vitamin D sterols, little information exists as to the relationship between PTH and indices of bone turnover and mineralization in children with mild and moderate stages of CKD.

The characterization of the actions of fibroblast growth factor 23 (FGF-23) has led to a new conceptual framework in understanding of the pathogenesis and treatment of CKD-MBD, with increasing levels in patients with CKD likely maintaining normophosphatemia at the expense of declining 1,25(OH)2vitamin D values (6). Previous studies in adult and pediatric patients have demonstrated that FGF-23 levels are elevated in early CKD and increase with CKD progression, suggesting that renal injury affects the skeleton in even very early stages of CKD (7–9). However, the skeletal changes associated with circulating FGF-23 levels in predialysis CKD are unknown, and it remains uncertain whether FGF-23 levels increase before PTH elevations become detectable or whether an increase in PTH, which has been shown in some studies to increase FGF-23 levels (10), precedes the increase in FGF-23. Thus, this study was designed to characterize the features of renal osteodystrophy and its relationship to biochemical markers of mineral metabolism in pediatric predialysis CKD.

Materials and Methods

Patients 2–21 years of age with stable stage 2–4 CKD, along with those with stage 5 CKD who were not yet on dialysis, were candidates for iliac crest
bone biopsy. Anthropometric parameters along with blood determinations of creatinine, bicarbonate, calcium, albumin, phosphorus, alkaline phosphatase, 25(OH)vitamin D, and 1,25(OH)2vitamin D, PTH, and FGF-23 were obtained at the time of bone biopsy. GFR was estimated according to the formula developed by Schwartz et al. (11). All children had documented kidney disease (renal scarring, non-nephrotic range proteinuria, and/or a GFR <60 ml/min per 1.73 m2), and CKD stage was determined according to recommendations from the National Kidney Foundation (12). Patients treated with growth hormone or immunosuppressive agents within the previous 6 months, those with nephrotic range proteinuria, and those who had undergone parathyroidectomy within the preceding year were excluded. This study was approved by the UCLA Human Subject Protection Committee and informed consent was obtained from all parents and/or patients.

Bone Histomorphometry

Patients were admitted to the UCLA General Clinical Research Center and full-thickness bone biopsies (0.5 cm in diameter by 1–2 cm in length) were obtained from the anterior iliac crest using a modified Bordier trephine needle after double tetracycline labeling (13). Specimens were dehydrated in alcohol, cleared with xylene, and embedded in methylmethacrylate. Primary bone histomorphometric parameters were assessed in trabecular bone under ×200 magnification using the OsteoMetrics system (OsteoMetrics, Decatur, GA) by a histomorphometrist (R.C.P.) blinded to biochemical values and CKD stage. Static histomorphometric parameters were evaluated in undecalcified 5-µm sections treated with Toluidine blue stain; tetracycline labeling was assessed in unstained 10-µm sections. Mineralized bone was defined by dark-blue staining areas; pale-blue seams at 1.5 µm in width were included in measurements of osteoid. Derived indices were calculated as described by Parfitt et al. (14). Normal parameters of bone histomorphometry were defined from bone biopsies obtained from a control group of 31 pediatric patients with normal kidney function (mean age, 12.4±1.5 years; 71% male; 48% Caucasian and 26% Hispanic) undergoing elective orthopedic surgery (13).

Biochemical Determinations

Serum calcium, phosphorus, albumin, creatinine, and alkaline phosphatase values were measured using an Olympus AU5400 analyzer (Olympus America Inc, Center Valley, PA). PTH concentrations in EDTA plasma were measured by the first-generation immunometric assay (Immutopsics, San Clemente, CA) (normal range, 10–65 pg/ml). FGF-23 levels were determined in EDTA plasma by a second-generation C-terminal assay (Immutopsics). The normal range (<100 RU/ml) was defined by Isakova et al. (9) and confirmed in a cohort of 26 healthy children (12±3 years of age). RIA was used to measure 25(OH)vitamin D and 1,25(OH)2vitamin D levels (15).

Statistical Analysis

Determinations of bone parameters and biochemical variables are reported as mean±SD or median (interquartile range). Skewed values were log-transformed before statistical analysis. ANOVA was used to assess differences in biochemical and bone histomorphometric parameters between the stages of CKD. Pearson correlation coefficients and multiple linear regression analysis were used to express the relationship between bone histomorphometric variables and biochemical parameters. Estimated GFR, calcium, phosphorus, alkaline phosphatase, 1,25(OH)2vitamin D, 25(OH)vitamin D, PTH, and FGF-23 were considered as potential covariates in predicting serum/plasma biochemical values and bone formation rate. Bone formation rate and all biochemical parameters were considered covariates in the prediction of all other bone histomorphometric variables. Interactions between potential predictors were evaluated. All statistical analyses were performed using SAS software (SAS Institute Inc, Cary, NC) and all tests were two sided. A probability of type I error <5% was considered statistically significant, and ordinary P values are reported.

Results

Patient Demographics and Biochemical Parameters

Fifty-eight patients (33 male and 25 female participants, 13.3±4.4 years of age, with an average GFR of 39±26 ml/min per 1.73 m2) were eligible for the study, and 52 patients (30 male and 22 female participants) 12.2±5.2 years of age underwent bone biopsy. CKD in study participants was due to obstructive or reflux nephropathy (n=32), glomerular disease (n=9), cystic disease (n=5), nephrocalcinosis (n=4), or unknown etiology (n=2). Of the 52 patients who enrolled, 31 participants were Hispanic, 18 were Caucasian, and 3 were black. Thirteen patients (CKD stage 2, n=1; CKD stage 3, n=8; and CKD stage 4, n=4) were treated with daily oral calcitriol, whereas 6 of these 13 patients were also treated with phosphate binders (CKD stage 3, n=3; and CKD stage 4, n=3) before bone biopsy. The average Z scores for height and weight were −1.33±1.4 and −0.61±1.53, respectively, and values did not differ by CKD stage. Biochemical parameters of study participants by CKD stage are displayed in Table 1. Serum calcium levels were within the normal range in all participants, whereas phosphorus concentrations were elevated only in patients with CKD stages 3 (4%) and 4/5 (43%).

Alkaline phosphatase levels were within the normal range and values did not differ between CKD stages. PTH levels were within the normal range in the majority of patients with CKD stage 2 but were elevated in a significant percentage in more advanced stages of CKD (Figure 1, Table 1). FGF-23 levels were increased in 81% of all patients, regardless of CKD stage (Figure 1, Table 1). Bicarbonate levels were <22 mEq/L (3) in 43%–70% of patients in each CKD stage. The 25(OH)vitamin D values were <30 ng/dl (3) in 62% but were <20 ng/dl (16) in only 13% of participants. Serum 1,25(OH)2vitamin D concentrations were in the normal range in early CKD stages but were low in more advanced CKD stages.

In multivariable analysis, GFR, calcium, and phosphorus were independent predictors of PTH (adjusted R2=0.61). No biochemical parameters were predictors of 1,25(OH)2vitamin D concentrations; however, 25(OH) vitamin D, phosphorus, and the interaction between GFR and PTH were independent predictors of plasma
received phosphate binders or vitamin D sterols) and then reconstituted patients with CKD stage 4 (Table 1). Only four patients from the majority of participants with CKD stage 2 and 3, whereas rates were above the normal range in 29% of patients with CKD stage 2 and in 86% of patients with CKD stage 4/5. Similarly, prolongation in osteoid maturation time was observed in 43% of patients with stage 2 CKD, 79% with CKD stage 3, and 79% with CKD stage 4/5. To separate a true mineralization defect from hyperostoidosis resulting from increased osteoblastic activity, “defective mineralization” was defined as an increase in osteoid volume/bone volume in combination with a prolongation in osteoid maturation time. Twenty-nine percent of all patients with stage 2 CKD, 42% with stage 3 CKD, and 79% with stage 4/5 CKD displayed evidence of a mineralization defect (Figure 1).

Overall, the majority of patients, irrespective of CKD stage, displayed either normal or increased bone volume, and only two patients (one with stage 1 CKD and one with stage 2 CKD) had evidence of low bone volume.

Table 1. Biochemical parameters across the spectrum of CKD

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Stage 2 CKD (n=14)</th>
<th>Stage 3 CKD (n=24)</th>
<th>Stage 4/5 CKD (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.5±0.4</td>
<td>9.2±0.5</td>
<td>9.3±0.9</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.7±0.8</td>
<td>4.7±1.0</td>
<td>6.1±1.2</td>
</tr>
<tr>
<td>Bicarbonate (mEq/L)</td>
<td>22.2±3.1</td>
<td>21.8±5.1</td>
<td>19.7±2.0</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>238±82</td>
<td>238±161</td>
<td>246±135</td>
</tr>
<tr>
<td>25(OH) vitamin D (µg/ml)</td>
<td>31.2±9.3</td>
<td>25.2±8.0</td>
<td>32.6±13.4</td>
</tr>
<tr>
<td>1,25(OH)2 vitamin D (pg/ml)</td>
<td>39.5±13.3</td>
<td>34.5±12.6</td>
<td>26.6±18.6</td>
</tr>
<tr>
<td>PTH (pg/ml), median (interquartile range)</td>
<td>52 (48, 87)</td>
<td>92 (46, 142)</td>
<td>125 (88, 366)</td>
</tr>
<tr>
<td>FGF-23 (RU/ml), median (interquartile range)</td>
<td>181 (101, 291)</td>
<td>197 (120, 300)</td>
<td>344 (255, 742)</td>
</tr>
</tbody>
</table>

FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone.

Values are expressed as mean ± SD unless otherwise noted.

Relationship between Biochemical Parameters and Bone Variables

**Bone Turnover.** Although circulating values of phosphorus and PTH were related to bone turnover in bivariate analysis (r=0.31, P<0.05; and r=0.28, P<0.05, respectively), FGF-23 was the sole independent predictor of bone formation rate in multivariable analysis. However, the predictive capability of even this parameter in the prediction of bone formation rate was poor (adjusted R²=0.15).

**Mineralization.** In bivariate analysis, circulating values of PTH, alkaline phosphatase, and calcium were each directly related to osteoid accumulation (r=0.38, P<0.05; r=0.46, P<0.01; and r=−0.28, P<0.05, respectively). In multivariable analysis, only bone formation rate, PTH, and phosphorus retained independent significance (adjusted R²=0.43). Serum phosphorus was the sole independent predictor of osteoid maturation time (adjusted R²=0.32).

To characterize the factors associated with the development of abnormal mineralization, patients in each CKD stage were grouped according to those with normal mineralization (Table 3) versus those with abnormal mineralization.

**Bone Histology.** Bone histomorphometric variables are displayed according to CKD stage in Table 2. Bone turnover was normal in the majority of participants with CKD stage 2 and 3, whereas rates were above the normal range in 29% of patients with CKD stage 4 (Table 1). Only four patients from the entire cohort—two with stage 2 CKD (neither of whom received phosphate binders or vitamin D sterols) and two with stage 3 (both of whom received oral calcitriol)—displayed evidence of adynamic bone.

Abnormalities in skeletal mineralization were present as early as CKD stage 2 (Figure 1). Excess osteoid accumulation, defined as increased osteoid volume/bone volume, was observed in 43% of patients with CKD stage 2 and in 86% of patients with CKD stage 4/5. Similarly, prolongation in osteoid maturation time was observed in 43% of patients with stage 2 CKD, 79% with CKD stage 3, and 79% with CKD stage 4/5. To separate a true mineralization defect from hyperostoidosis resulting from increased osteoblastic activity, “defective mineralization” was defined as an increase in osteoid volume/bone volume in combination with a prolongation in osteoid maturation time. Twenty-nine percent of all patients with stage 2 CKD, 42% with stage 3 CKD, and 79% with stage 4/5 CKD displayed evidence of a mineralization defect (Figure 1).

FGF-23 concentrations (adjusted R²=0.67). FGF-23 levels were directly associated with height Z score in both bivariate analysis (r=0.54, P<0.01) and multivariable analysis (adjusted R²=0.26).

Bone histomorphometric variables are displayed according to CKD stage in Table 2. Bone turnover was normal in the majority of participants with CKD stage 2 and 3, whereas rates were above the normal range in 29% of patients with CKD stage 4 (Table 1). Only four patients from the entire cohort—two with stage 2 CKD (neither of whom received phosphate binders or vitamin D sterols) and two with stage 3 (both of whom received oral calcitriol)—displayed evidence of adynamic bone.

Abnormalities in skeletal mineralization were present as early as CKD stage 2 (Figure 1). Excess osteoid accumulation, defined as increased osteoid volume/bone volume, was observed in 43% of patients with CKD stage 2 and in 86% of patients with CKD stage 4/5. Similarly, prolongation in osteoid maturation time was observed in 43% of patients with stage 2 CKD, 79% with CKD stage 3, and 79% with CKD stage 4/5. To separate a true mineralization defect from hyperostoidosis resulting from increased osteoblastic activity, “defective mineralization” was defined as an increase in osteoid volume/bone volume in combination with a prolongation in osteoid maturation time. Twenty-nine percent of all patients with stage 2 CKD, 42% with stage 3 CKD, and 79% with stage 4/5 CKD displayed evidence of a mineralization defect (Figure 1).

Overall, the majority of patients, irrespective of CKD stage, displayed either normal or increased bone volume, and only two patients (one with stage 1 CKD and one with stage 2 CKD) had evidence of low bone volume.

FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone.

Values are expressed as mean ± SD unless otherwise noted.

P<0.05 above the normal range.

P<0.05 from stage 2 and stage 3 CKD.
Calculating PTH levels (93). Patients with normal mineralization had lower cir-
mentation; FGF-23 leve ls did not differ between 
mineralization despite serum bicarbonate concentrations 

mained normal across all CKD stages. Bone turnover was 
although calcium and alkalin e phosphatase levels re-

Discussion 
This study demonstrated that increases in plasma FGF-23 
values occurred in the majority of patients in stage 2 CKD, 
all but one of whom were naïve of therapy with active 

Volume. No biochemical parameters were predictors of 

Table 2. Bone histomorphometric variables across the spectrum of CKD

<table>
<thead>
<tr>
<th>Variableb</th>
<th>Stage 2 CKD (n=14)</th>
<th>Stage 3 CKD (n=24)</th>
<th>Stage 4/5 CKD (n=14)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnover</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bone formation rate (BFR/BS) (μm³/μm² per day)</td>
<td>24.9±4.2</td>
<td>39.9±35.9</td>
<td>64.2±56.0c</td>
<td>8.0–73.4</td>
</tr>
<tr>
<td>eroded surface (ES/BS) (%)</td>
<td>5.2±0.6</td>
<td>4.7±3.8</td>
<td>9.0±7.7</td>
<td>0.5–4.3</td>
</tr>
<tr>
<td>Mineralization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>osteoid volume (OV/BV) (%)</td>
<td>4.8±4.5</td>
<td>8.6±8.8</td>
<td>9.8±5.0c,d</td>
<td>0.2–5.8</td>
</tr>
<tr>
<td>osteoid thickness (O.Th) (μm)</td>
<td>9.2±3.2</td>
<td>14.2±5.9c</td>
<td>14.0±3.3c</td>
<td>2.0–13.2</td>
</tr>
<tr>
<td>osteoid surface (OS/BS) (%)</td>
<td>27.0±16.1</td>
<td>35.4±19.6</td>
<td>44.6±15.9d</td>
<td>4.3–37.0</td>
</tr>
<tr>
<td>osteoid maturation time (OMT) (d)</td>
<td>11.1±4.4</td>
<td>17.9±9.5c</td>
<td>16.6±7.6</td>
<td>1.2–11.5</td>
</tr>
<tr>
<td>mineralization lag time (MLT) (d)</td>
<td>54.0±47.4</td>
<td>63.3±66.3</td>
<td>66.5±71.3</td>
<td>2.3–63.8</td>
</tr>
<tr>
<td>mineral apposition rate (MAR) (μm/d)</td>
<td>0.91±0.37</td>
<td>0.86±0.30</td>
<td>0.97±0.39</td>
<td>1.1–1.5</td>
</tr>
<tr>
<td>adjusted apposition rate (Aj.Ar) (μm/d)</td>
<td>0.35±0.37</td>
<td>0.35±0.25</td>
<td>0.37±0.25</td>
<td>0.14–1.20</td>
</tr>
<tr>
<td>mineralized surface/bone surface (MS/BS) (%)</td>
<td>7.4±3.8</td>
<td>11.6±8.2</td>
<td>17.7±12.8</td>
<td>2.2–19.0</td>
</tr>
<tr>
<td>mineralized surface/bone volume (MS/BV) (%)</td>
<td>48.3±69.4</td>
<td>42.4±34.1</td>
<td>39.2±22.4</td>
<td>7.3–66.2</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bone volume (BV/TV) (%)</td>
<td>23.1±7.8</td>
<td>27.8±7.4</td>
<td>27.4±8.9</td>
<td>8.9–34.4</td>
</tr>
<tr>
<td>trabecular thickness (Tb.Th) (μm)</td>
<td>122±26</td>
<td>136±34</td>
<td>142±29</td>
<td>91–175</td>
</tr>
<tr>
<td>trabecular number (Tb.N) (#/μm)</td>
<td>1.9±0.5</td>
<td>2.0±0.3</td>
<td>1.9±0.4</td>
<td>1.1–2.2</td>
</tr>
<tr>
<td>trabecular separation (Tb.Sp) (μm)</td>
<td>452±169</td>
<td>381±97</td>
<td>416±174</td>
<td>351–737</td>
</tr>
</tbody>
</table>

aValues are expressed as mean ± SD unless otherwise noted. 
bThe formulae used to calculate derived bone histomorphometric indices from primary measurements are presented next to each variable. Additional details are provided in Supplemental Material. 
cP<0.05 from stage 2 CKD. 
dP<0.05 from stage 2 and stage 3 CKD.

| Table 3. Biochemical values associated with normal mineralization by CKD stage

<table>
<thead>
<tr>
<th>Biochemical Value</th>
<th>Stage 2 CKD (n=10)</th>
<th>Stage 3 CKD (n=14)</th>
<th>Stage 4/5 CKD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)b</td>
<td>9.6±0.4</td>
<td>9.3±0.3</td>
<td>10.0±0.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.6±0.7</td>
<td>4.9±0.9</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td>Bicarbonate (mEq/L)</td>
<td>21.8±3.3</td>
<td>21.5±3.9</td>
<td>22.0±1.0</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>232±91</td>
<td>203±98</td>
<td>242±237</td>
</tr>
<tr>
<td>25(OH) vitamin D (ng/ml)</td>
<td>30.7±10.4</td>
<td>27.1±7.1</td>
<td>33.5±3.7</td>
</tr>
<tr>
<td>1,25(OH)₂ vitamin D (pg/ml)</td>
<td>40.0±15.5</td>
<td>31.3±10.9</td>
<td>24.4±22.6</td>
</tr>
<tr>
<td>PTH (pg/ml)c,d</td>
<td>66±41; 54 (48, 87)</td>
<td>74±36; 72 (42, 101)</td>
<td>273±370; 104 (19, 698)</td>
</tr>
<tr>
<td>FGF-23 (RU/ml)c</td>
<td>218±140; 200 (101, 291)</td>
<td>240±180; 197 (83, 431)</td>
<td>543±281; 543 (344, 742)</td>
</tr>
</tbody>
</table>

FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone. 
aValues are expressed as mean ± SD unless otherwise noted. 
bP<0.05 for parameter between patients with normal and abnormal mineralization. 
cValues include mean ± SD and median (interquartile range).
predialysis CKD, and PTH levels were only weakly correlated with bone turnover. By contrast, defective mineralization was present as early as stage 2 CKD and its prevalence increased with progressive CKD stages. Defective skeletal mineralization was associated with lower serum calcium levels and increased PTH concentrations.

An understanding of the interplay between PTH, FGF-23, Klotho, phosphorus, and vitamin D is critical for advancing the still limited understanding of the pathogenesis and future treatment of CKD-MBD. In this study, as well as in a recently published large cohort of adults with predialysis CKD (9), circulating FGF-23 levels were already elevated in stage 2 CKD, consistent with the marked upregulation of FGF-23 expression in bone observed in patients with this degree of kidney disease (18). Although controversial (19–21), some investigators have shown that FGF-23 levels increase in response to oral phosphate loading in healthy volunteers (22) as well as in wild-type and CKD rats (23); thus, an increased, possibly intermittent, phosphate burden in the context of even mildly decreased renal function may contribute to enhanced FGF-23 secretion. Phosphate-independent mechanisms, such as subtle, subclinical elevations in PTH, may also contribute to the increase in FGF-23 levels in CKD. Indeed, recent animal and human data suggest that PTH, either directly or indirectly, stimulates FGF-23 secretion (10,24), although conditioned medium from calvarial cultures treated with PTH revealed no increase in FGF-23 levels (11). Circulating levels of FGF-23 have been identified as an independent risk factor for mortality in adult CKD patients and in the general population at large (25–27). In addition, short-term trials have demonstrated that treatment with either sevelamer hydrochloride or lanthanum carbonate may reduce elevated FGF-23 levels, resulting in increased circulating 1,25(OH)2 vitamin D and decreased PTH values without altering serum phosphorus levels in normophosphatemic patients with stage 3 and stage 4 CKD (6,28). Although further studies are required to assess the long-term effect of targeting phosphate burden and FGF-23 levels on the systemic complications associated with CKD-MBD, the prevention of excessive intestinal phosphate absorption in early CKD, when serum phosphorus levels are in the normal range, may thus be important in the early management of CKD-MBD (6). However, the association between FGF-23 and height Z score in this study, as well as previous observations that FGF-23 levels correlate with IGFI levels in children with CKD (8), suggest that therapies aimed at lowering FGF-23 levels may have consequences on growth in the pediatric age group.

Consistent with previous data from the adult population (29), this study also demonstrated that PTH levels were within the normal range in the majority of patients with stage 2 CKD and were increased above the normal range in 63% of patients with stage 3 and stage 4/5 CKD. At the same time, bone turnover was normal in all patients with stage 2 CKD and increased in only 18% of patients with stage 3 and stage 4/5 CKD, despite the lack of therapy with active vitamin D sterol in the majority of patients. Although a weak correlation existed between PTH and bone formation rate in bivariate analysis, this relationship was completely lost in multivariable analyses, and the ability of any biochemical parameters to predict bone turnover was very poor. Thus, reliance on PTH or on any other currently used biochemical parameter for the prediction of bone histology is inadvisable; bone biopsy remains the only currently reliable method for the assessment of bone turnover in pediatric patients with predialysis CKD.

Abnormalities in bone mineralization are highly prevalent in dialyzed children (30) and often persist after successful renal transplantation (13). The present study further demonstrates a high prevalence of skeletal mineralization defects even in early stages of CKD. These findings may be unique to the growing skeleton because the presence of mineralization defects is currently uncommon in adults treated with maintenance dialysis (31,32). Despite normal rates of bone formation in the vast majority of patients across the stages of CKD, mineralization abnormalities were present as early as stage 2 CKD, when serum levels of calcium and phosphorus were within the normal range. Acidemia, a condition associated with defective skeletal mineralization (17), was present in a substantial percentage of study participants; however, defective mineralization was equally prevalent in nonacidemic individuals, suggesting that acidemia did not affect the prevalence of abnormal mineralization in the current cohort. Moreover, levels of

Table 4. Biochemical values associated with abnormal mineralization as defined by an increase in osteoid volume and a delay in osteoid maturation time by CKD stage*  

<table>
<thead>
<tr>
<th>Biochemical Value</th>
<th>Stage 2 CKD (n=10)</th>
<th>Stage 3 CKD (n=14)</th>
<th>Stage 4/5 CKD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)b</td>
<td>9.2±0.4</td>
<td>9.2±0.7</td>
<td>9.1±0.9</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.8±1.1</td>
<td>4.4±1.1</td>
<td>6.2±1.3</td>
</tr>
<tr>
<td>Bicarbonate (mEq/L)</td>
<td>23.3±2.9</td>
<td>22.3±6.6</td>
<td>19.1±4.1</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>253±65</td>
<td>286±219</td>
<td>246±111</td>
</tr>
<tr>
<td>25(OH) vitamin D (ng/ml)</td>
<td>33.1±4.8</td>
<td>22.3±2.9</td>
<td>32.4±15.2</td>
</tr>
<tr>
<td>1,25(OH)2 vitamin D (pg/ml)</td>
<td>38.3±2.5</td>
<td>39.1±14.1</td>
<td>27.2±18.6</td>
</tr>
<tr>
<td>PTH (pg/ml)b,c</td>
<td>64±46; 52 (26, 115)</td>
<td>144±85; 153 (87, 188)</td>
<td>238±220; 145 (88, 366)</td>
</tr>
<tr>
<td>FGF-23 (RU/ml)c</td>
<td>162±100; 160 (100, 231)</td>
<td>247±176; 240 (120, 300)</td>
<td>469±525; 225 (111, 1072)</td>
</tr>
</tbody>
</table>

FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone.

*Values are expressed as mean ± SD unless otherwise noted.

bP<0.05 for parameter between patients with normal and abnormal mineralization.

Values include mean ± SD and median (interquartile range).
25(OH)vitamin D were >20 ng/ml—the target range, although controversial (33), currently recommended by the Institute of Medicine (16) in 87% of individuals and levels were not related to any parameter of bone histology. However, 25(OH)vitamin D values were <30 ng/ml (3)—the target recommended by the Kidney Disease Outcomes Quality Initiative—in 62% of patients. Langman et al. previously demonstrated that the mineralization defect in children with predialysis CKD was corrected by 25(OH) vitamin D therapy (34), suggesting that higher values may be necessary and 25(OH) vitamin D repletion may be important for optimal bone health in the pediatric CKD population.

Consistent with the stimulatory actions of PTH on osteoblasts, circulating PTH levels were associated with increased osteoid accumulation in this study and, consistent with data from Hyp mice (35), phosphorus concentrations were consistently, independently, and inversely associated with both osteoid accumulation and osteoid maturation time. These findings suggest that altered phosphorus sensing or metabolism may be involved in the pathogenesis of the defective skeletal mineralization in pediatric CKD. In contrast to findings in pediatric dialysis-dependent patients, the presence of abnormal mineralization in the current cohort was not directly related to circulating FGF-23 levels, suggesting that renal FGF-23 excretion may obscure the relationship between FGF-23 and bone histology in patients with predialysis CKD. Regardless of its etiology, the prevalence of defective mineralization with progressive CKD stages validates the current Kidney Disease Improving Global Outcomes recommendation for the assessment of renal osteodystrophy in patients across all CKD stages (36).

In conclusion, elevated circulating FGF-23 levels are present in very early stages of pediatric CKD, suggesting that an increased phosphate burden in the context of even mildly decreased renal function may contribute to enhanced FGF-23 secretion. Early defects in skeletal mineralization suggest that factors other than the traditional markers such as acidosis and 25(OH) vitamin D and mineral deficiency are altered early in the course of CKD and play a crucial role in the development of renal bone disease. Finally, biochemical parameters, including PTH, are poor predictors of bone turnover in pediatric patients with predialysis CKD and bone biopsy remains the gold standard for the assessment of renal osteodystrophy in this population.

Acknowledgments
This work was supported in part by grants from US Public Health Service (DK-67563, DK-35423, DK-51081, DK-073039, and RR-00865) and funds from the Casey Lee Ball Foundation.

This paper was presented in abstract form (SA-FC425) at the 2009 national meeting of the American Society of Nephrology.

Disclosures
H.J. is a named inventor on the patent outlining the development of immunometric assays for the detection of FGF-23.

References


12. Institute of Medicine: Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC, National Academy of Sciences, 2010


Received: June 18, 2011 Accepted: September 27, 2011

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.05940611/-/DCSupplemental.