Mineral Metabolism in European Children Living with a Renal Transplant: A European Society for Paediatric Nephrology/European Renal Association–European Dialysis and Transplant Association Registry Study

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Abstract

Background and objectives Data on mineral metabolism in pediatric renal transplant recipients largely arise from small single-center studies. In adult patients, abnormal mineral levels are related to a higher risk of graft failure. This study used data from the European Society for Paediatric Nephrology/European Renal Association–European Dialysis and Transplant Association Registry to study the prevalence and potential determinants of mineral abnormalities, as well as the predictive value of a disturbed mineral level on graft survival in a large cohort of European pediatric renal transplant recipients.

Design, setting, participants, & measurements This study included 1237 children (0–17 years) from 10 European countries, who had serum calcium, phosphorus, and parathyroid hormone measurements from 2000 onward. Abnormalities of mineral metabolism were defined according to European guidelines on prevention and treatment of renal osteodystrophy in children on chronic renal failure.

Results Abnormal serum phosphorus levels were observed in 25% (14% hypophosphatemia and 11% hyperphosphatemia), altered serum calcium in 30% (19% hypocalcemia, 11% hypercalcemia), and hyperparathyroidism in 41% of the patients. A longer time since transplantation was associated with a lower risk of having mineral levels above target range. Serum phosphorus levels were inversely associated with eGFR, and levels above the recommended targets were associated with a higher risk of graft failure independently of eGFR.

Conclusions Abnormalities in mineral metabolism are common after pediatric renal transplantation in Europe and are associated with graft dysfunction.

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Introduction

Abnormalities in calcium-phosphorus metabolism and consequent bone mineral disorders are a frequent and severe complication in children with ESRD (1). Maintaining normal mineral levels is important to not only ensure adequate linear growth (2) but to also avoid cardiovascular complications (3).

Although disturbances in mineral metabolism are highly prevalent in pediatric dialysis patients (4), mineral abnormalities generally improve after transplantation. However, altered mineral metabolism appears to persist in some patients (5) with a potential effect on osteoporosis (6) and graft function. A high phosphorus load might promote deposition of calcium-phosphate crystals in the renal tubules, leading to microvascular and interstitial calcifications, which may contribute to the risk of graft failure (7). Indeed, in children (8) and adult patients with early (9) or advanced CKD (10), serum phosphorus was independently associated with a decline in renal function. Moreover, donor (11), pretransplant (12,13), and post-transplant (14–16) mineral levels have been associated with graft failure in adult renal graft recipients. Information on mineral metabolism in pediatric graft recipients mainly originates from small single-center studies with relatively short follow-up time (5,17,18). Therefore, we aimed to study the prevalence and potential determinants of a disturbed mineral metabolism in a large cohort of European pediatric renal transplant patients. Moreover, we studied the effect of mineral levels on graft function.

Materials and Methods

Study Participants

Data on mineral levels were collected within the European Society for Paediatric Nephrology/European

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Renal Association–European Dialysis and Transplant Association (ESPN/ERA-EDTA) Registry. On an annual basis, the registry collects individual patient data on date of birth, sex, treatment modality at start of RRT, and subsequent modality changes of all European children requiring RRT. Furthermore, a variable set of anthropometric, clinical, and medication-related parameters is collected. For these analyses, all measurements on serum calcium, phosphorus, and parathyroid hormone (PTH) collected from 2000 onward from patients aged <18 years with a functioning graft were included. This included data from the following countries and periods: Belarus (2009–2010), Belgium (2010–2011), Denmark (2006–2011), Finland (2000–2011), Hungary (2010), Norway (2009–2011), Portugal (2008–2011), Slovenia (2007–2011), Turkey (2011–2012), and the United Kingdom (2002–2012).

Definition of Variables

All biochemical variables (*e.g.*, serum creatinine and mineral levels) are determined according to local practice. Because we included measurements from 2000 onward, we assumed serum creatinine levels were determined with the enzymatic method. The eGFR was calculated with the new bedside Schwartz equation (19). Abnormalities in calcium, phosphorus, and PTH were assessed using European Pediatric Dialysis Working Group (EPDWG) guidelines on prevention and treatment of renal osteodystrophy in children on chronic renal failure (20) (Table 1). Although these guidelines are largely opinion based, they take into account the age dependency of mineral metabolism during childhood. Calcium levels were assumed to be uncorrected for serum albumin and were therefore corrected as follows: calcium (milligrams per decaliter)+0.8 (4 − serum albumin [grams per decaliter]). Calcium and phosphorus levels are age dependent. Therefore, to make meaningful comparisons across the pediatric age range, we calculated SD scores using pediatric reference values (21). Missing values for albumin (18.6%), height (13.2%), and serum creatinine (2.2%) were imputed using multiple imputation creating five imputed data sets, thereby taking into account the uncertainty in the measurements (22,23). To test the validity of the Schwartz eGFR using imputed heights, we performed sensitivity analyses calculating eGFR according to the height-independent equation of Pottel *et al.* (24), which showed similar associations between mineral metabolism and eGFR according to both equations.

Table 1. Target levels for PTH, calcium, and phosphate according to European guidelines on prevention and treatment of renal osteodystrophy in children with chronic renal failure

Variable	Target Level
PTH (pg/ml)	
GFR (ml/min per 1.73 m^2)	
>59	10–65
29< GFR≤59	10–65
15≤ GFR≤29	130–195
<15	130–195
Calcium (mg/dl)	
Age (yr)	
0–2	8.8–11.3
3–5	9.4–10.8
6–12	9.4–10.3
13–18	8.8–10.2
Phosphate (mg/dl)	
Age (yr)	
0–2	4.8–7.4
3–5	4.5–6.5
6–12	3.6–5.8
13–18	2.3–4.5

PTH, parathyroid hormone.

Statistical Analyses

To satisfy normality assumptions, PTH values were log-transformed. Because mineral levels were repeatedly measured within the same patient, we used multinomial generalized estimating equation models (25) with clusters on country and patient level and an autoregressive working correlation structure to estimate the prevalence of, and factors associated with, mineral levels outside target range. Risk of graft failure was calculated as hazard ratios (HRs) using time-dependent Cox proportional hazards regression models adjusting for late entry into the risk set. To investigate the confounding effect of low eGFR on the association between mineral levels and risk of graft failure, we performed two sensitivity analyses: one excluding eGFR measurements below 20 ml/min per 1.73 m^2 and one using marginal structural models. Marginal structural models adjust for bias caused by time-varying confounders (*i.e.*, eGFR) that may affect changes in the parameter of interest (mineral level) (26). We adjusted all analyses for potential confounders according to criteria for confounding (27). Variables included in the adjusted analyses were as follows: age, sex, year of transplantation, time since transplantation, and eGFR. P values <0.05 were considered statistically significant. All statistical analyses were performed in SAS software (version 9.3; SAS Institute Inc, Cary, NC).

Results

Description

Data on mineral levels were available for 1237 renal transplant patients from 10 different countries, contributing to a total of 3358 measurements (median 2; range, 1–12 measurements per patient). Median time since transplantation was 3.0 years (interquartile range, 1.1–6.2 years). Most patients were male (59.9%), started RRT on peritoneal dialysis (52.7%), and received a kidney from a deceased donor (55.6%). Congenital anomalies of the kidney and the urinary tract were the most common cause of renal failure (44.4%). At time of measurement, most patients were between 13 and 17 years of age (58.9%) and had a median eGFR of 62 ml/min per 1.73 m^2 (interquartile range, 49–78). Overall, the mean SD score for calcium was −0.07±1.16, whereas the mean phosphorus SD score was −0.50±0.98 (Table 2).

Mean height SD score in the total cohort was −1.87±0.79. The annual change in height SD score (*e.g.*, longitudinal growth) could be calculated for 678 patients. We did not find any association between mineral levels and annual change in height SD score. Mean annual change in height SD score was −0.02 (95% confidence interval [95% CI], −0.04 to 0.00) per unit change in calcium SD score, 0.02

Table 2. Patient characteristics

Characteristic	Patients (n=1237), n (%)	Measurements (n=3358)
Age at start of RRT (yr)		
0–2	402 (32.5)	1416
3–5	185 (15.0)	464
6–12	407 (32.9)	1021
13–17	243 (19.6)	457
Age at measurement (yr)		
0–2	35 (2.9)	81
3–5	112 (9.1)	335
6–12	361 (29.1)	1047
13–17	729 (58.9)	1895
Sex (%)		
Male	741 (59.9)	2033
Female	496 (40.1)	1325
Treatment modality at start of RRT		
Hemodialysis	265 (21.4)	619
Peritoneal dialysis	651 (52.7)	2024
Transplantation	317 (25.6)	709
Unknown/missing	4 (0.3)	6
Primary renal disease		
Congenital anomalies of the kidney and the urinary tract	549 (44.4)	1354
GN	114 (9.2)	240
Cystic kidneys	128 (10.4)	363
Hereditary nephropathy	182 (14.7)	757
Ischemic renal failure	26 (2.1)	62
Hemolytic-uremic syndrome	36 (2.9)	91
Metabolic disorders	39 (3.1)	80
Vasculitis	14 (1.1)	33
Miscellaneous	79 (6.4)	217
Unknown/missing	70 (5.7)	161
Transplant type		
Deceased donor	688 (55.6)	1971
Living donor	454 (36.7)	1153
Unknown donor	95 (7.7)	234
eGFR (ml/min per 1.73 m^2)		
Median (interquartile range)	62 (49, 78)	
≥90	153 (12.4)	388
60–89	529 (42.7)	1370
45–59	313 (25.3)	920
30–44	169 (13.7)	487
≤29	73 (5.9)	193
Calcium (mg/dl)		
Mean (SD)	9.56 (0.37)	
SD score, mean (SD)	−0.07 (1.16)	
Phosphorus (mg/dl)		
Mean (SD)	4.03 (0.50)	
SD score, mean (SD)	−0.50 (0.98)	
PTH, mean (SD) (pg/ml)	111 (162)	
Height SD score, mean (SD)	−1.87 (0.79)	

PTH, parathyroid hormone.

(95% CI, −0.01 to 0.05) per unit change in phosphorus SD score, and −0.001 (95% CI, −0.001 to 0.00) per unit change in PTH.

Abnormalities in Mineral Metabolism

Hypocalcemia was found in 19.0% of the patients, with the highest prevalence observed among patients aged 6–12 years (30.1%), whereas 11.1% of the patients showed hypercalcemia and this prevalence was lower after a longer period since transplantation (Figure 1). Prevalence of hypophosphatemia was 13.9%, whereas prevalence of hyperphosphatemia was 11.0%. The rate of hypophosphatemia was higher among infants (66.9%) than in patients aged 13–17 years (4.6%). Furthermore, prevalence of hypophosphatemia was highest in the early post-transplant period and among patients with lower eGFR levels, whereas the reverse was true for hyperphosphatemia (Figure 1). PTH levels were below target range in 5.2% and above target range in 40.9% of the patients. This pattern was similar for all age groups. Results indicated that 63.6% of the patients showed elevated PTH levels in the immediate post-transplant period,

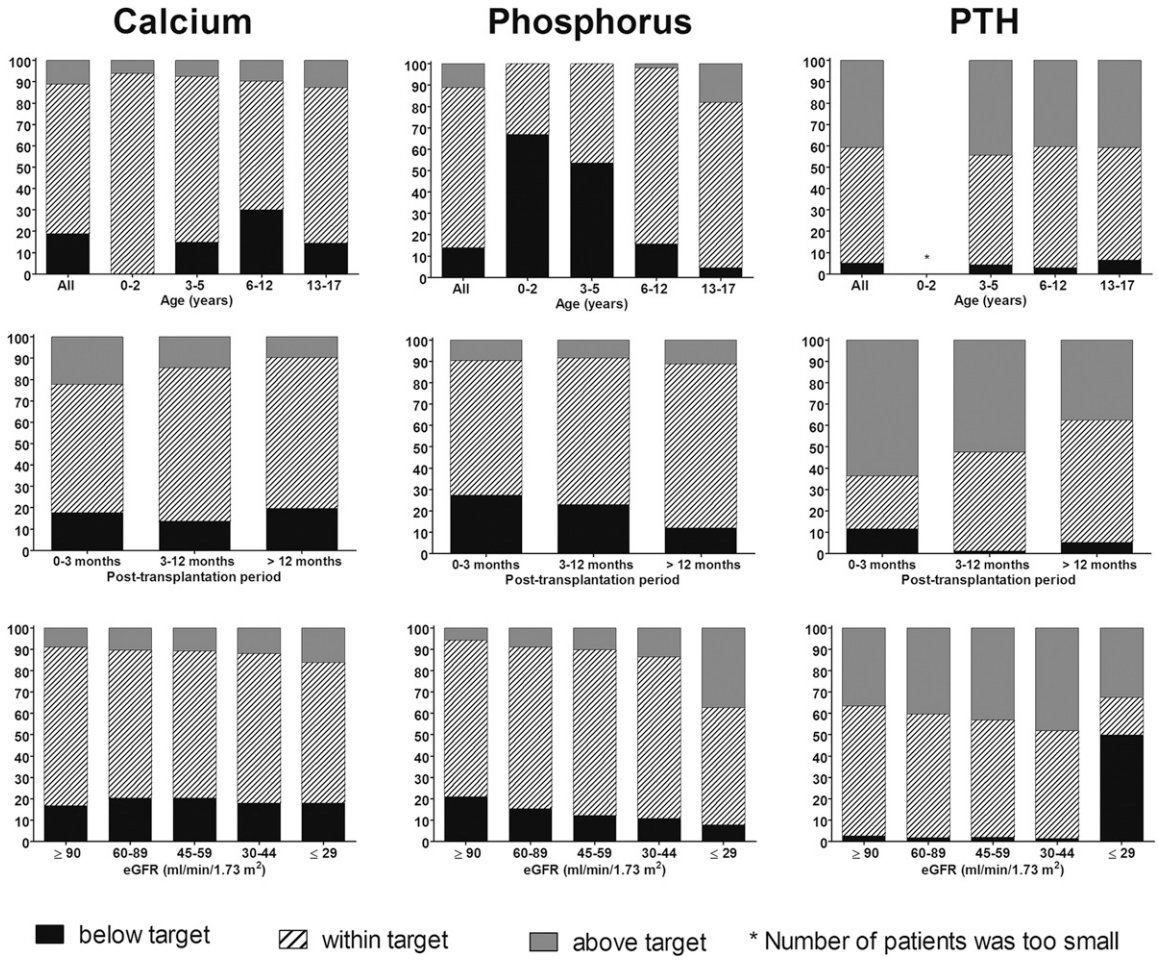


Figure 1. | Distribution of patients with calcium, phosphorus, and PTH levels according to target levels stratified by age, post-transplant period, and eGFR. The asterisk indicates that the number of patients was too small. PTH, parathyroid hormone.

whereas this was 37.6% after a post-transplant period of ≥12 months. The number of patients with PTH levels above the recommended target was higher with lower eGFR, except for the lowest eGFR group, in which 49.9% and 32.6% of the patients showed PTH levels below and above target range, respectively. However, this might partly be explained by the eGFR dependency of the PTH target levels (Figure 1).

Factors Associated with Mineral Levels Outside Target Range

Female sex was associated with a significantly lower risk of having serum calcium levels below target (odds ratio [OR], 0.76, 95% CI, 0.60 to 0.97) and serum phosphorus levels above target (OR, 0.60; 95% CI, 0.45 to 0.80) (Table 3). Among female adolescents (13–17 years), the lower risk of hyperphosphatemia was even stronger (OR, 0.51; 95% CI, 0.38 to 0.68). Longer time since transplantation was associated with a lower risk of having calcium and PTH levels above target range, and with a lower risk of subtarget phosphorus levels. Furthermore, patients who were transplanted preemptively had a lower risk of displaying mineral levels above target range compared with patients who had been on dialysis before receiving a renal transplant, but this was statistically significant only for serum calcium (OR, 0.43; 95% CI, 0.28 to 0.64) and PTH (OR, 0.64; 95% CI, 0.48 to 0.86). Spending a longer time on dialysis before transplantation was not associated with higher risks of having mineral levels outside the target range.

Patients who were off steroids showed a significantly lower risk of hypocalcemia compared with patients using steroids (OR, 0.41; 95% CI, 0.21 to 0.83), whereas patients using tacrolimus (OR, 3.37; 95% CI, 1.78 to 6.38) or patients not using calcineurin inhibitors as part of their immunosuppressive regimen (OR, 5.71; 95% CI, 2.85 to 11.42) showed a significantly higher risk of hypocalcemia compared with patients using cyclosporine.

Association between Mineral Levels and eGFR

Calcium SD score was not associated with eGFR, whereas phosphorus SD score and PTH levels were significantly higher in patients in the lowest eGFR group (eGFR≤29 ml/min per 1.73 m^2) compared with patients with an eGFR>90 ml/min per 1.73m^2 (Figure 2).

Mineral Levels and Risk of Graft Failure

HRs for the association between mineral levels and risk of graft failure are shown in Table 4. After a median follow-up of 3.0 years, 45 grafts were lost (including seven deaths).

Table 3. ORs for having mineral levels outside target range

Variable	Calcium		Phosphate		PTH	
	Low	High	Low	High	Low	High
Age (yr)[a]						
0–2	—	—	—	—	—	—
3–5	0.47 (0.34 to 0.65)[b]	0.68 (0.43 to 1.08)	—	—	1.76 (0.67 to 4.63)	1.11 (0.71 to 1.73)
6–12	1.00	1.00	1.00	1.00	1.00	1.00
13–17	0.43 (0.34 to 0.53)[b]	1.35 (1.04 to 1.76)[b]	0.28 (0.20 to 0.38)[b]	10.08 (6.15 to 16.53)[b]	2.46 (1.29 to 4.68)[b]	0.98 (0.75 to 1.28)
Sex						
Female	0.76 (0.60 to 0.97)[b]	1.25 (0.95 to 1.65)	0.86 (0.66 to 1.12)	0.60 (0.45 to 0.80)[b]	0.73 (0.40 to 1.34)	1.05 (0.81 to 1.37)
Male	1.00	1.00	1.00	1.00	1.00	1.00
Time on transplantation (yr)[c]						
<0.5	0.39 (0.26 to 0.61)[b]	3.05 (1.64 to 5.69)[b]	1.80 (1.06 to 3.06)[b]	1.45 (0.84 to 2.48)	0.31 (0.06 to 1.78)	3.26 (1.93 to 5.51)[b]
0.5 < 1	0.50 (0.34 to 0.75)[b]	2.65 (1.64 to 4.29)[b]	0.96 (0.50 to 1.82)	1.38 (0.77 to 2.43)	0.25 (0.05 to 1.35)	1.88 (1.15 to 3.06)[b]
1 < 2.5	0.65 (0.50 to 0.87)[b]	1.17 (0.70 to 1.96)	0.73 (0.47 to 1.14)	1.33 (0.89 to 1.98)	0.42 (0.15 to 1.15)	1.61 (1.08 to 2.39)[b]
≥2.5	1.00	1.00	1.00	1.00	1.00	1.00
Preemptive transplant[c]						
Yes	1.54 (1.17 to 2.02)[b]	0.43 (0.28 to 0.64)[b]	0.88 (0.62 to 1.25)	0.72 (0.50 to 1.04)	1.37 (0.71 to 2.63)	0.64 (0.48 to 0.86)[b]
No	1.00	1.00	1.00	1.00	1.00	1.00
Time on dialysis before transplant (yr)[c]						
<0.25	1.03 (0.51 to 2.05)	1.92 (0.97 to 3.77)	0.24 (0.06 to 0.92)[b]	0.93 (0.50 to 1.71)	—	—
0.25 < 1	0.80 (0.56 to 1.14)	0.96 (0.65 to 1.41)	1.08 (0.70 to 1.68)	1.03 (0.72 to 1.49)	1.06 (0.44 to 2.54)	1.02 (0.68 to 1.50)
1 < 2	0.87 (0.61 to 1.23)	1.29 (0.88 to 1.88)	1.10 (0.70 to 1.72)	0.69 (0.45 to 1.05)	0.94 (0.40 to 2.24)	0.79 (0.55 to 1.13)
≥2	1.00	1.00	1.00	1.00	1.00	1.00
Steroids[d]						
Yes	1.00	1.00	1.00	1.00	—	—
No	0.41 (0.20 to 0.83)[b]	1.43 (0.83 to 2.46)	0.60 (0.26 to 1.35)	1.56 (0.86 to 2.81)	—	—
Calcineurin inhibitors[d]						
Cyclosporin A	1.00	1.00	1.00	1.00	—	—
Tacrolimus	2.34 (1.54 to 3.55)[b]	0.35 (0.22 to 0.56)[b]	1.07 (0.73 to 1.57)	1.09 (0.67 to 1.77)	—	—
None	5.71 (2.85 to 11.42)[b]	0.16 (0.05 to 0.54)[b]	1.09 (0.33 to 3.66)	1.53 (0.74 to 3.16)		

ORs are presented with 95% CIs. Dashes indicate that the number of patients with levels below or above target range in this category was too low to obtain an effect estimate. OR, odds ratio; 95% CI, 95% confidence interval; PTH, parathyroid hormone.

[a]Adjusted for sex.

[b]Significantly different from 1.

[c]Adjusted for age, sex, and year of transplantation.

[d]Adjusted for age, sex, time since transplantation, year of transplantation, and eGFR.

Although not significantly different, higher calcium levels (and calcium SD score) were associated with a lower risk of graft failure. Calcium levels below EPDWG target range were independently associated with a higher risk of graft failure compared with levels within target range (HR, 2.45; 95% CI, 1.10 to 5.47); however, after additional adjustment for eGFR, this association was no longer statistically significant. Phosphorus levels were independently associated with a higher risk of graft failure (adjusted HR per 0.30 mg/dl, 1.19; 95% CI, 1.07 to 1.33), but this association lost its statistical significance after further adjustment for eGFR. Because phosphorus levels within target range are possibly safe, we studied the effect of phosphorus levels above target. They were associated with a higher risk of graft failure compared with having phosphorus levels within target range, and this association remained after adjustment for eGFR (adjusted HR, 2.18; 95% CI, 1.10 to 4.32). To test the confounding effect of low eGFR levels in this association, we performed two sensitivity analyses. Our first sensitivity analysis, excluding eGFR measurements below 20 ml/min per 1.73 m^2, showed that after adjustment for age, sex, year of transplantation, and eGFR phosphorus levels above EPDWG target range were associated with a higher risk of graft failure, but this association was not statistically significant (HR, 1.68; 95% CI, 0.78 to 3.60). The second sensitivity analysis, using marginal structural models and thereby adjusting for the effect of eGFR on phosphorus levels but not for the effect of phosphorus levels on eGFR, showed a significantly higher risk of graft failure for phosphorus levels above target range (HR, 2.04; 95% CI, 1.19 to 3.49).

PTH levels below target range were significantly associated with graft failure (adjusted HR, 10.3; 95% CI, 2.77 to 38.13). However, this seemed mainly the effect of the eGFR dependency of PTH target levels, because the effect was no longer statistically significant after additional adjustment for eGFR.

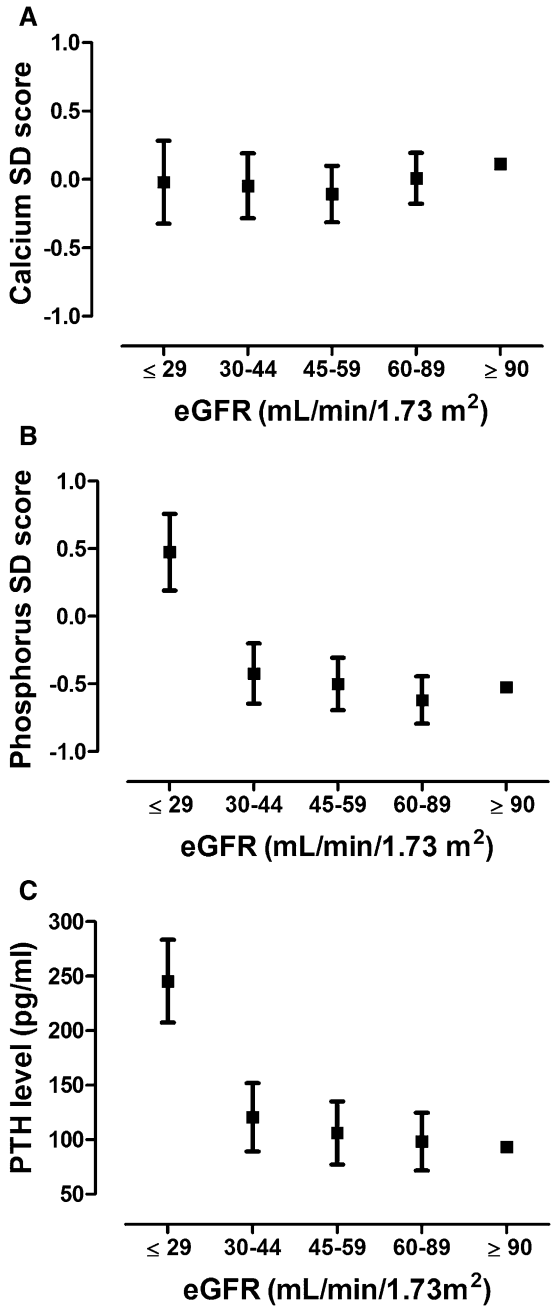


Figure 2. | **Mineral levels according to eGFR**. Data represent levels of calcium SD score (A), phosphorus SD score (B), and PTH (C) with 95% confidence intervals. Adjustments were made for age, sex, time since transplantation, and year of transplantation. PTH, parathyroid hormone.

Discussion

In this study, we demonstrate that disturbances in mineral metabolism are frequent in pediatric renal graft recipients. Furthermore, we found significant inverse associations between eGFR and phosphorus and PTH levels, as well as a higher risk of graft failure in patients with levels of serum phosphorus above the recommended targets.

We found a high prevalence of abnormalities in calcium, phosphorus, and PTH levels. Abnormalities in phosphorus levels were largely age dependent. Confirming previous reports (5,28,29), we observed that 25% of the patients had phosphorus levels outside target range. Hypophosphatemia was more prevalent than hyperphosphatemia, ranging from 67% in infants to 5% in adolescents. A high prevalence of hypophosphatemia (41%) has also been noted in infants on dialysis (4) and has been related to insufficient dietary supply. Hence, many patients may already be in a phosphate-depleted state when undergoing transplantation. In the early post-transplant period, impaired phosphorus reabsorption due to tubular dysfunction of the allograft may aggravate hypophosphatemia. Persistently elevated PTH and possibly fibroblast growth factor-23 (FGF-23) secretion may also contribute to urinary phosphate losses after transplantation (5). Although serum FGF-23 and vitamin D levels are not reported to our registry, our data show lower rates of hyperparathyroidism after a period of transplantation. Because hypophosphatemia may affect the mineralization of the growing bone, our findings point to a need for regular monitoring of serum phosphorus levels and consequent phosphate supplementation of hypophosphatemic patients.

Overall, 41% of our patients had elevated PTH levels. The prevalence of hyperparathyroidism was lower after a longer time since transplantation. When applying the same guidelines, the prevalence of hyperparathyroidism in our

Table 4. HRs for mineral levels and graft failure

Mineral Level	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[a]	Adjusted HR (95% CI)[b]
Calcium			
Continuous per 0.40 mg/dl	0.85 (0.71 to 1.03)	0.83 (0.68 to 1.02)	0.90 (0.74 to 1.09)
SD score, per SD score	0.85 (0.70 to 1.03)	0.83 (0.67 to 1.01)	0.92 (0.76 to 1.12)
EPDWG targets			
Below target	2.25 (1.02 to 4.96)[c]	2.45 (1.10 to 5.47)[c]	1.50 (0.66 to 3.41)
Within target	1.00	1.00	1.00
Above target	0.66 (0.26 to 1.67)	0.63 (0.25 to 1.61)	0.73 (0.29 to 1.85)
Phosphorus			
Continuous per 0.30 mg/dl	1.19 (1.07 to 1.32)[c]	1.19 (1.07 to 1.33)[c]	1.08 (0.96 to 1.20)
SD score, per SD score	1.33 (1.12 to 1.60)[c]	1.34 (1.12 to 1.60)[c]	1.11 (0.93 to 1.33)
EPDWG targets			
Below target	0.76 (0.16 to 3.65)	0.75 (0.16 to 3.61)	0.67 (0.14 to 3.24)
Within target	1.00	1.00	1.00
Above target	3.79 (1.96 to 7.33)[c]	3.82 (1.97 to 7.42)[c]	2.18 (1.10 to 4.32)[c]
PTH			
Continuous per 10 pg/ml	1.01 (1.00 to 1.02)	1.01 (0.99 to 1.02)	1.00 (0.98 to 1.01)
EPDWG targets			
Below target	7.76 (2.20 to 27.4)[c]	10.3 (2.77 to 38.13)[c]	2.14 (0.49 to 9.34)
Within target	1.00	1.00	1.00
Above target	1.45 (0.52 to 4.01)	1.44 (0.51 to 4.07)	0.87 (0.27 to 2.74)

EPDWG guidelines on prevention and treatment of renal osteodystrophy in children on chronic renal failure. HR, hazard ratio; 95% CI, 95% confidence interval; EPDWG, European Pediatric Dialysis Working Group; PTH, parathyroid hormone.
[a]Adjusted for age, sex, and year of transplantation.
[b]Adjusted for age, sex, eGFR, and year of transplantation.
[c]Significantly different from 1.

study is lower compared with North America (5) and Iran (28) (both 57%), and might reflect regional differences in PTH control, in keeping with data from pediatric patients on peritoneal dialysis (4).

Children who were on dialysis before transplantation tended to have higher mineral levels than those transplanted preemptively, possibly due to the persistence of hyperparathyroidism (5,12,30). However, we did not find an association between dialysis vintage and mineral disturbances. Furthermore, a longer time since transplantation was associated with a lower risk of having mineral levels above target range, suggesting that mineral levels improve over time, in keeping with others (30). This potential improvement might also be explained by a more optimal management over time. With a mean height SD score of −1.87, growth failure was highly prevalent after pediatric renal transplantation. However, there was no association between mineral levels and annual change in height SD score, suggesting that other factors play a more dominant role in growth after transplantation.

Surprisingly, we found a lower risk of hyperphosphatemia among female patients, especially among adolescents. Data from a recent study in adults suggest that women are more susceptible to hyperactivity of the parathyroid gland due to estrogen action (30). Unfortunately, information regarding estrogen levels or menstrual history was not available. Immunosuppressive drugs can induce mineral effects. Corticosteroids can modulate calcium homeostasis, leading to decreased intestinal absorption and increased urinary excretion (31). Indeed, patients off steroids had a significantly lower risk of hypocalcemia compared with patients using steroids.

We found inverse relationships of serum phosphorus and PTH levels with renal function. Levels were found relatively stable up to 30 ml/min per 1.73 m^2 eGFR and were significantly higher at lower eGFR. By contrast, we did not find any association with global renal function for calcium. This is in line with other studies, as Bachetta *et al.* did not find any relationship between GFR and phosphorus and calcium among patients with GFRs>30 ml/min per 1.73 m^2 (32). In studies in native kidneys, normal phosphorus levels, phosphorus SD score, and PTH were maintained until GFR dropped below 30 (33), whereas serum calcium was independent of GFR.

We found an association between serum phosphorus and risk of graft failure. Several studies have shown associations between serum phosphorus levels and decline in renal function in pediatric and adult patients with CKD (8–10), and graft failure in adult renal transplant patients (11,13–15). Serum phosphorus levels above recommended targets were associated with a higher risk of graft loss, even after adjustment for eGFR. Because a decreasing eGFR affects overall patient well-being, increasing serum phosphorus levels might also be a marker of disease severity, and elevated phosphorus levels are thus rather the consequence of the failing transplant. It should be noted that the patient group (5.9% of total population) with the lowest eGFR (<29 ml/min per 1.73 m^2) is responsible for the statistical weight of this association. When excluding this group, the association between serum phosphorus and graft failure lost its significance. This is not surprising, because statistical power is markedly reduced when the patient group with the highest risk of graft loss is excluded. Similarly, in our sensitivity

analysis excluding eGFR levels below 20 ml/min per 1.73 m^2, the association between elevated phosphorus levels and graft failure lost its significance. The effect in this sensitivity analysis is likely to be a long-term effect as the mean time between the measurement of high phosphorus and graft failure was 2.6 years. Our other sensitivity analysis (the marginal structural model), likewise identified a higher risk of graft failure for elevated phosphorus levels, suggesting that phosphorus levels may be the cause of graft failure. However, our study is observational and can therefore not prove causation. By contrast, two studies among adult (16) and pediatric (5) graft recipients did not show consistent associations between elevated phosphorus levels and decrease of renal function, time to first rejection, graft loss, or mortality. They did, however, find independent associations of elevated FGF-23 with these outcomes, even in a subgroup of patients with eGFR between 30–90 (16). This difference might be due to the longer follow-up time in our study, because recent data in children with predialysis CKD have shown that FGF-23 concentrations increased early and progressively as GFR declined, and preceded any increase in serum phosphorus (34). Unfortunately, FGF-23 is not reported in our registry, so we were not able to test whether this hormone was associated with graft failure. More research is needed to elucidate whether, and by which pathophysiological mechanisms, serum phosphorus levels may lead to a higher risk of graft failure.

Although we studied a large population with a long follow-up, and used repeatedly measured mineral levels for estimating the risk of graft failure instead of using only a single baseline value, some limitations of our work need to be acknowledged. These include the lacking availability of serum vitamin D or FGF-23 levels, which might have provided greater insight into potential etiological mechanisms. In addition, the reported medication use (*e.g.*, phosphate or vitamin D supplementation) or mineral intake was very limited in our registry; therefore, we were not able to adjust the associations for medication use. Phosphorus levels seem to follow the normal circadian rhythm (35) and fluctuate during the day. In this large patient population, the variation in measurements is likely to be leveled out. Furthermore, the use of different analytical methods for determining mineral levels may have influenced our results. PTH measurements in particular are subject to high intermethod variability (36). However, the association between mineral levels and outcomes is likely unrelated to varying mineral levels due to timing of measurement or use of different analytical methods (nondifferential misclassification), which, if anything, would most likely have led to an underestimation or dilution of the reported effects.

To summarize, abnormalities in mineral metabolism after pediatric kidney transplantation are common. In infants, hypophosphatemia is highly prevalent, reflecting persistently impaired bone mineralization, even after transplantation, during this critical period of growth and development. Conversely, adolescent graft recipients are largely hyperphosphatemic. Hyperphosphatemia predicts the loss of graft function, although cause-effect relationships are unclear.

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Disclosures

None.

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