Nutrition and growth in European children with end-stage renal disease

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MINERAL METABOLISM IN EUROPEAN CHILDREN LIVING WITH A RENAL TRANSPLANT: AN ESPN/ERA-EDTA REGISTRY STUDY

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Background: Data on mineral metabolism in paediatric renal transplant recipients are scarce. In adult patients abnormal mineral levels are related to an increased risk of graft failure. We used data from the ESPN/ERA-EDTA registry to study the prevalence and potential determinants of mineral abnormalities, and the predictive value of a disturbed mineral level on graft survival in European paediatric renal transplant recipients.

Methods: We included 1237 children (0-17 years) with serum calcium, phosphorus and parathyroid hormone (PTH) measurements from the year 2000 onwards from ten European countries. Abnormalities of mineral metabolism were defined according to the guidelines of the European Paediatric Dialysis Working Group.

Results: Abnormal serum phosphorus levels were observed in 25% (14% hypo- and 11% hyperphosphatemia), altered serum calcium in 30% (19% hypo-, 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 an increased risk of graft failure independently of eGFR

Conclusion: Abnormalities in mineral metabolism are common after paediatric renal transplantation in Europe and are associated with graft dysfunction.

ABSTRACT

INTRODUCTION

Abnormalities in calcium-phosphorus metabolism and consequent bone mineral disorders are a frequent and severe complication in children with end-stage renal disease (ESRD) [1]. Maintaining normal mineral levels is not only important to ensure adequate linear growth [2], but also to avoid cardiovascular complications [3].
While disturbances in mineral metabolism are highly prevalent in paediatric dialysis patients [4], mineral abnormalities generally improve after transplantation. However, altered mineral metabolism appears to persist in some patients [5] with potential impact on graft function. A high phosphorus load might promote deposition of calcium-phosphate crystals in the renal tubules, leading to micro-vascular and interstitial calcifications which may contribute to the risk of graft failure [6]. Indeed, in children [7] and adult patients with early [8] or advanced chronic kidney disease [9], serum phosphorus was independently associated with a decline in renal function. Moreover, donor [10], pre-transplant [11,12], and post-transplant [13,14] mineral levels have been associated with graft failure in adult renal graft recipients. Information on mineral metabolism in paediatric graft recipients is, however, scarce.
Therefore, we aimed to study the prevalence and potential determinants of a disturbed mineral metabolism in a cohort of European paediatric renal transplant patients. Moreover, we studied the effect of mineral levels on graft function.

METHODS

Subjects

Data on mineral levels were collected within the registry of the European Society of Paediatric Nephrology and the European Renal Association and European Transplant Association, the ESPN/ERA-EDTA registry. On an annual basis, the registry collects individual patient data on date of birth, gender, treatment modality at start of RRT, and subsequent modality changes of all European children requiring renal replacement therapy (RRT). Furthermore, a variable set of data on anthropometric, clinical and medication-related parameters is collected. For the present analyses, all measurements on serum calcium, phosphorus, and PTH from patients with a functioning graft aged less than 18 years collected from the year 2000 onwards 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

Abnormalities in calcium, phosphorus, parathyroid hormone (PTH) and calcium x phosphorus (Ca x P) product were assessed using target levels established by the European Paediatric Dialysis Working Group (EPDWG) [15]. Although these guidelines are largely opinion-based and refer to peritoneal dialysis patients, they take into account the age-dependency of mineral metabolism during childhood. Target ranges are shown in Table 1. We assumed that reported calcium levels were uncorrected for serum albumin, and we therefore corrected serum calcium levels using the following formula: calcium (mmol/l) + 0.02 (40- serum albumin (g/l)). The Ca x P product in mg^2/dl^2 was calculated by multiplying corrected calcium (in mg/dl) and phosphorus (in mg/dl) levels. Calcium and phosphorus levels are age-dependent. Therefore, to make meaningful comparisons across the paediatric age range, we calculated SDS using paediatric reference values [16]. Estimated glomerular filtration rate (eGFR) was calculated using the new bedside Schwartz formula [17]. Missing values for albumin (18.6%), height (13.2%), and serum creatinine (2.2%) were imputed using multiple imputation creating five imputed datasets, thereby taking into account the uncertainty in the measurements [18,19]. To test the validity of Schwartz eGFR using imputed heights, we performed sensitivity analyses calculating eGFR according to the height-independent equation of Pottel *et al.* [20]. These analyses showed similar associations between mineral metabolism and eGFR according to both equations.

Table 1. Target levels for PTH, calcium, phosphate and Ca x P product according to EPDWG guidelines

PTH (pg/ml)	
GFR > 59 ml/min/1.73m^2	10-65
29 < GFR ≤ 59 ml/min/1.73m^2	10-65
15 ≤ GFR ≤ 29 ml/min/1.73m^2	130-195
GFR < 15 ml/min/1.73m^2	130-195
Calcium (mmol/l)	
0-2 years	2.20-2.83
3-5 years	2.35-2.70
6-12 years	2.35-2.60
13-18 years	2.20-2.55
Phosphate (mmol/l)	
0-2 years	1.55-2.39
3-5 years	1.45-2.10
6-12 years	1.16-1.87
13-18 years	0.74-1.45
Ca x P product (mg^2/dl^2)	
0-12 years	< 55
13-18 years	< 60

Statistical analyses

To satisfy normality assumptions, PTH values were log-transformed. As mineral levels were repeatedly measured within the same patient, we used multinomial generalized estimating equation (GEE) models [21] 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 the target range. The risk of graft failure was calculated as hazard ratios using time-dependent Cox proportional hazards regression models adjusting for late entry into the risk set. To test whether mineral levels were the cause rather than the consequence of the failing transplant, we performed two sensitivity analyses. One excluding the eGFR measurements below 20 ml/min/1.73m^2 and one using marginal structural models. Marginal structural models adjust for the bias caused by time-varying confounders (i.e. eGFR) that may affect changes in the parameter of interest (mineral level) [22]. We adjusted all analyses for potential confounders according to criteria for confounding [23]. *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Description

Patient characteristics are summarized in Table 2. Data on mineral levels were available for 1237 renal transplant patients from ten different countries, contributing to a total of 3358 measurements (median 2, range 1-12 measurements per patient). The median time since transplantation was 3.0 years (IQR: 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 (CAKUT) were the most common cause of renal failure (44.4%). At the time of measurement, most patients were between 13 and 17 years of age (58.9%) and had a median eGFR of 62 [IQR: 49-78] ml/min/1.73 m^2. The mean standard deviation score (SDS) for calcium in the total cohort was -0.07 ± 0.03, whereas the mean phosphorus SDS was -0.50 ± 0.03.

Table 2. Patient characteristics

	Patients (N=1237) (3358 Measurements)
Age at start of RRT	**N (%)**
0-2 years	402 (32.5)
3-5 years	185 (15.0)
6-12 years	407 (32.9)
13-17 years	243 (19.6)
Age at measurement	
0-2 years	35 (2.9)
3-5 years	112 (9.1)
6-12 years	361 (29.1)
13-17 years	729 (58.9)
Gender (%)	
Male	741 (59.9)
Female	496 (40.1)
Treatment modality at start of RRT	
HD	265 (21.4)
PD	651 (52.7)
Tx	317 (25.6)
Unknown/Missing	4 (0.3)
Primary renal disease	
CAKUT	549 (44.4)
Glomerulonephritis	114 (9.2)
Cystic Kidneys	128 (10.4)
Hereditary Nephropathy	182 (14.7)
Ischemic Renal Failure	26 (2.1)
HUS	36 (2.9)
Metabolic Disorders	39 (3.1)
Vasculitis	14 (1.1)
Miscellaneous	79 (6.4)
Unknown/missing	70 (5.7)
Transplant type	
Deceased donor	688 (55.6)
Living donor	454 (36.7)
Unknown donor	95 (7.7)
Median eGFR [IQR] (ml/min/1.73 m^2)	62 [49; 78]
eGFR ≥ 90 ml/min/1.73 m^2	153 (12.4)
eGFR 60-89 ml/min/1.73 m^2	529 (42.7)
eGFR 45-59 ml/min/1.73 m^2	313 (25.3)
eGFR 30-45 ml/min/1.73 m^2	169 (13.7)
eGFR ≤ 29 ml/min/1.73 m^2	73 (5.9)
Mean calcium (SE)(mmol/l)	2.39 (0.003)
Mean calcium SDS (SE)	-0.07 (0.03)
Mean phosphorus (SE) (mmol/l)	1.30 (0.01)
Mean phosphorus SDS (SE)	-0.50 (0.03)
Mean PTH (SE) (pg/ml)	111 (6)

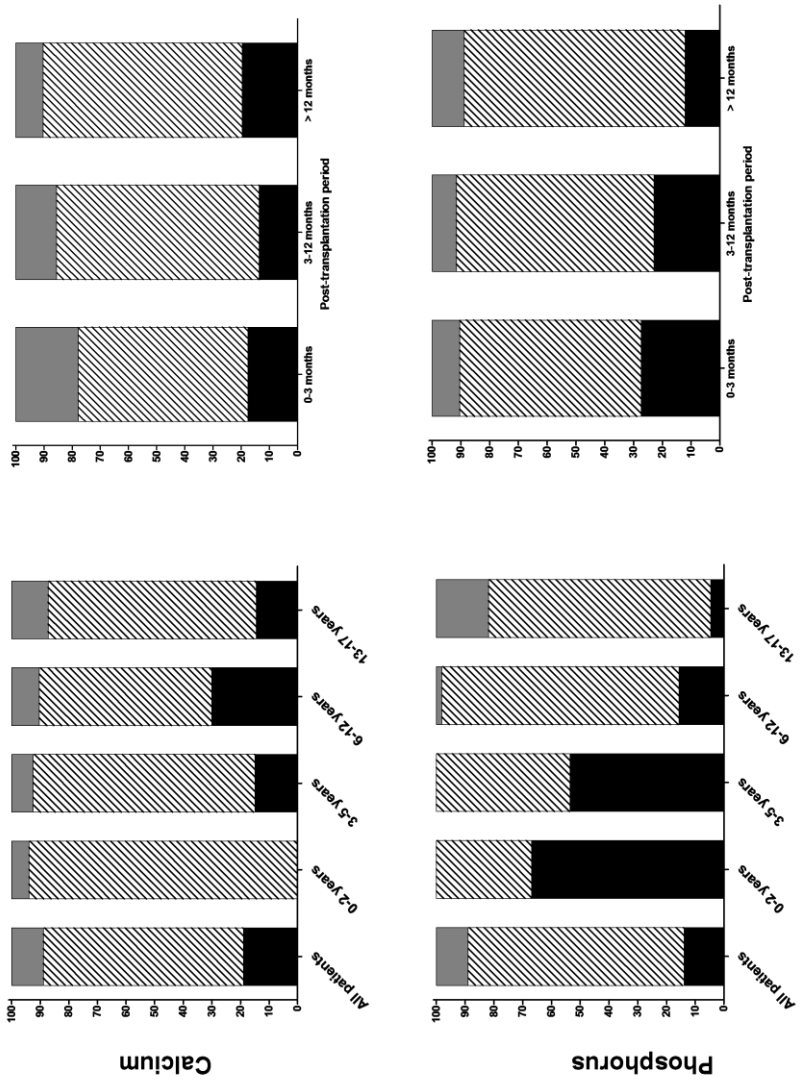

Figure 1A. Distribution of patients with calcium and phosphorus levels according to target levels stratified by age and post-transplant period

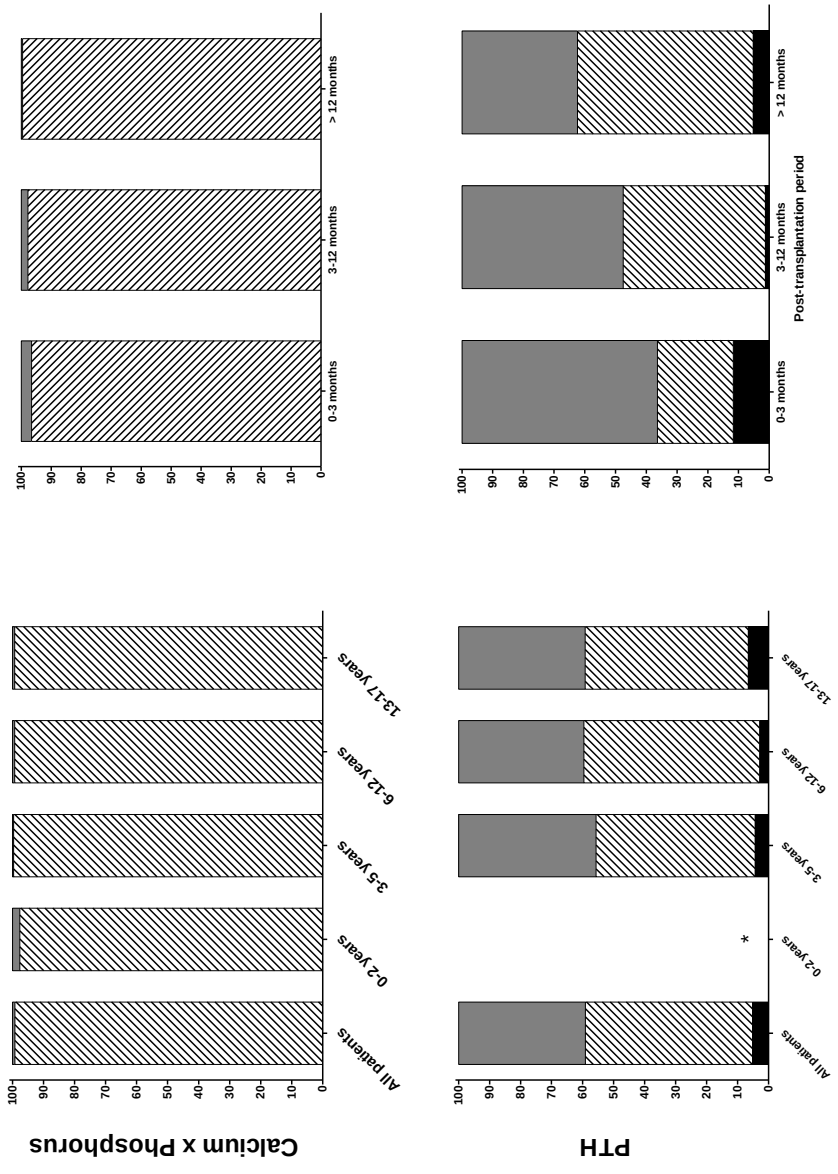

Figure 1B. Distribution of patients with Ca x P product and PTH levels according to target levels stratified by age and post-transplant period

* The number of subjects was too small

Abnormalities in mineral metabolism

Percentages of patients with serum mineral levels below, within, and above target levels according to EPDWG stratified by age and post-transplant period are depicted in Figure 1A and 1B. Hypocalcaemia was found in 19.0% of the patients, with the highest prevalence observed among 6-12 year olds (30.1%), whereas 11.1% of the patients showed hypercalcemia and this prevalence decreased with time after transplantation (Figure 1A). The prevalence of hypophosphatemia was 13.9%, whereas the prevalence of hyperphosphatemia was 11.0%. The rate of hypophosphatemia decreased with age from 66.9% in infants to 4.6% in the 13-17 year old patients and with time since transplantation, whereas for hyperphosphatemia the reverse was true (Figure 1A). In all age groups, calcium-phosphorus (Ca x P) product was within target range for 99% of the patients. Parathyroid hormone (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. PTH levels improved with time since transplantation, with 63.6% of the patients showing elevated levels in the immediate post-transplant period decreasing to 37.6% after a post-transplant period of 12 months or more (Figure 1B).

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: 0.60-0.97) and serum phosphorus levels above target (OR: 0.60, 95% CI: 0.45-0.80) (Table 3). Among female adolescents (13-17 years) the lower risk of hyperphosphatemia was even stronger (OR: 0.51, 95% CI: 0.38-0.68). A longer time since transplantation was associated with a lower risk of having calcium, Ca x P product and PTH levels above target range, and with a lower risk of sub-target phosphorus levels. Furthermore, patients who were transplanted pre-emptively had a lower risk of displaying mineral levels above target range as compared to patients who spent some time on dialysis before receiving a renal transplant, but this was statistically significant only for serum calcium (OR: 0.43, 95% CI: 0.28-0.64) and PTH (OR: 0.64, 95% CI: 0.48-0.86). Spending a longer time on dialysis prior to transplantation was not associated with higher risks of having mineral levels outside the target range.

Patients who were off steroids showed a significantly decreased risk of hypocalcaemia as compared to patients using steroids (OR: 0.41, 95% CI: 0.21-0.83), while patients using tacrolimus (OR: 3.37, 95% CI: 1.78-6.38) or patients not using calcineurin inhibitors as part of their immunosuppressive regimen (OR: 5.71, 95% CI: 2.85-11.42) showed a significant increased risk of hypocalcaemia as compared to patients using cyclosporine.

Table 3. Odds Ratios for having mineral levels outside target range

	Calcium		Phosphate		Ca x P	PTH	
	low	high	low	high	high	low	high
Age[1]							
0-2 years	#	#	**#**	#	#	#	#
3-5 years	**0.47 (0.34-0.65)**	0.68 (0.43-1.08)	**#**	**#**	0.67 (0.18-2.46)	1.76 (0.67-4.63)	1.11 (0.71-1.73)
6-12 years	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13-17 years	**0.43 (0.34-0.53)**	**1.35 (1.04-1.76)**	**0.28 (0.20-0.38)**	**10.08 (6.15-16.53)**	0.90 (0.40-2.01)	**2.46 (1.29-4.68)**	0.98 (0.75-1.28)
Sex							
Female	**0.76 (0.60-0.97)**	1.25 (0.95-1.65)	0.86 (0.66-1.12)	**0.60 (0.45-0.80)**	0.88 (0.36-2.16)	0.73 (0.40-1.34)	1.05 (0.81-1.37)
Male	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Time on Tx[2]							
< 0.5 years	**0.39 (0.26-0.61)**	**3.05 (1.64-5.69)**	**1.80 (1.06-3.06)**	1.45 (0.84-2.48)	**4.99 (1.30-19.23)**	0.31 (0.06-1.78)	**3.26 (1.93-5.51)**
0.5 < 1 years	**0.50 (0.34-0.75)**	**2.65 (1.64-4.29)**	0.96 (0.50-1.82)	1.38 (0.77-2.43)	**5.38 (1.50-19.29)**	0.25 (0.05-1.35)	**1.88 (1.15-3.06)**
1 < 2.5 years	**0.65 (0.50-0.87)**	1.17 (0.70-1.96)	0.73 (0.47-1.14)	1.33 (0.89-1.98)	**0.61 (0.10-3.79)**	0.42 (0.15-1.15)	**1.61 (1.08-2.39)**
≥ 2.5 years	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pre-emptive Tx[2]							
Yes	**1.54 (1.17-2.02)**	**0.43 (0.28-0.64)**	0.88 (0.62-1.25)	0.72 (0.50-1.04)	0.28 (0.04-2.18)	1.37 (0.71-2.63)	**0.64 (0.48-0.86)**
No	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Time on dialysis prior to Tx[2]							
< 0.25 years	1.03 (0.51-2.05)	1.92 (0.97-3.77)	**0.24 (0.06-0.92)**	0.93 (0.50-1.71)	0.70 (0.10-4.76)	#	#
0.25 < 1 years	0.80 (0.56-1.14)	0.96 (0.65-1.41)	1.08 (0.70-1.68)	1.03 (0.72-1.49)	0.83 (0.31-2.22)	1.06 (0.44-2.54)	1.02 (0.68-1.50)
1 < 2 years	0.87 (0.61-1.23)	1.29 (0.88-1.88)	1.10 (0.70-1.72)	0.69 (0.45-1.05)	0.44 (0.13-1.55)	0.94 (0.40-2.24)	0.79 (0.55-1.13)
≥ 2 years	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Steroids[3]							
Yes	1.00	1.00	1.00	1.00	1.00	#	#
No	**0.41 (0.20-0.83)**	1.43 (0.83-2.46)	0.60 (0.26-1.35)	1.56 (0.86-2.81)	2.54 (0.58; 13.45)	#	#
Calcineurin inhibitors[3]							
Cyclosporine A	1.00	1.00	1.00	1.00	#	#	#
Tacrolimus	**2.34 (1.54-3.55)**	**0.35 (0.22-0.56)**	1.07 (0.73-1.57)	1.09 (0.67-1.77)	#	#	#
None	**5.71 (2.85-11.42)**	**0.16 (0.05-0.54)**	1.09 (0.33-3.66)	1.53 (0.74-3.16)	#	#	#

[1]Adjusted for sex; [2]Adjusted for age, sex, and year of transplantation; 3 Adjusted for age, sex, time since transplantation, year of transplantation, and eGFR #Number of patients with levels below or above target range in this category was too low to obtain an effect estimate

Association between mineral levels and eGFR

The association between mineral levels and eGFR is shown in Figure 2. Calcium SDS was not associated with eGFR, whereas phosphorus SDS, Ca x P product and PTH levels were all significantly higher in patients in the lowest eGFR group (eGFR ≤ 29 mL/min/1.73m^2) compared to patients with an eGFR > 90 mL/min/1.73m^2.

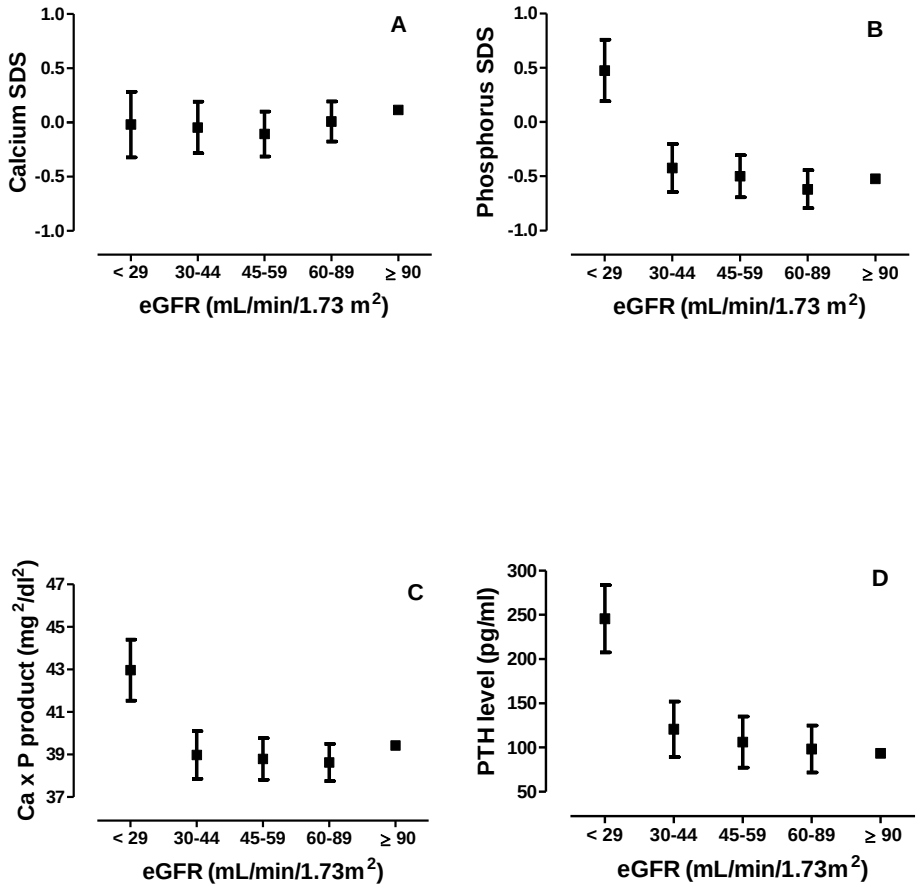

Figure 2. Association between eGFR and mean levels of calcium SDS (A), phosphorus SDS (B), Ca x P product (C), and PTH (D)
Adjustments were made for age, sex, time since transplantation, and year of transplantation

Mineral levels and risk of graft failure

Hazard ratios (HR) 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 7 deaths). Although not significantly different, higher calcium levels (and calcium SDS) 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 as compared to levels within target range (HR: 2.45, 95% CI: 1.10-5.47), but 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 0.10 mmol/l: 1.19 95% CI: 1.07-1.33), but also this association lost its statistical significance after further adjustment for eGFR. As 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 to having phosphorus levels within the target range, and this association remained after adjustment for eGFR (adjusted HR: 2.18, 95% CI: 1.10-4.32). To test whether phosphorus levels were the cause rather than the consequence of the failing transplant we performed two sensitivity analyses. Our first sensitivity analysis, excluding the eGFR measurements below 20 mL/min/1.73m^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: 0.78-3.60). The second sensitivity analysis, using marginal structural models, 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: 1.19-3.49).

After adjustment for age, sex, and year of transplantation, each 1 mg^2/dl^2 increase in Ca x P product was associated with a 5% higher risk of graft failure (adjusted HR: 1.05, 95% CI: 1.01-1.09). After further adjustment for eGFR this association was no longer statistically significant (Table 4). PTH levels below target range were significantly associated with graft failure (adjusted HR: 10.3, 95% CI: 2.77-38.13). However, this seemed mainly the effect of the eGFR dependency of PTH target levels, as the effect was no longer statistically significant after additional adjustment for eGFR.

Table 4. Hazard Ratios (HRs) for mineral levels and graft failure

	Unadjusted HR (95% CI)	Adjusted HR[1] (95% CI)	Adjusted HR[2] (95% CI)
Calcium continuous per 0.10 mmol/l increase	0.85 (0.71-1.03)	0.83 (0.68-1.02)	0.90 (0.74-1.09)
Calcium SDS per SDS increase	0.85 (0.70-1.03)	0.83 (0.67-1.01)	0.92 (0.76-1.12)
EPDWG targets calcium			
below target	**2.25 (1.02-4.96)**	**2.45 (1.10-5.47)**	1.50 (0.66-3.41)
within target	1.00	1.00	1.00
above target	0.66 (0.26-1.67)	0.63 (0.25-1.61)	0.73 (0.29-1.85)
Phosphorus continuous per 0.10 mmol/l increase	**1.19 (1.07-1.32)**	**1.19 (1.07-1.33)**	1.08 (0.96-1.20)
Phosphorus SDS per SDS increase	**1.33 (1.12-1.60)**	**1.34 (1.12-1.60)**	1.11 (0.93-1.33)
EPDWG targets phosphorus			
below target	0.76 (0.16-3.65)	0.75 (0.16-3.61)	0.67 (0.14-3.24)
within target	1.00	1.00	1.00
above target	**3.79 (1.96-7.33)**	**3.82 (1.97-7.42)**	**2.18 (1.10-4.32)**
Ca x P product continuous	**1.05 (1.01-1.09)**	**1.05 (1.01-1.09)**	1.02 (0.98-1.06)
PTH continuous per 10 pg/ml increase	1.01 (1.00-1.02)	1.01 (0.99-1.02)	1.00 (0.98-1.01)
EPDWG targets PTH			
below target	**7.76 (2.20-27.4)**	**10.3 (2.77-38.13)**	2.14 (0.49-9.34)
within target	1.00	1.00	1.00
above target	1.45 (0.52-4.01)	1.44 (0.51-4.07)	0.87 (0.27-2.74)

[1]Adjusted for age, sex, year of transplantation [2]Adjusted for age, sex, eGFR, year of transplantation

DISCUSSION

In this study we demonstrate that disturbances in mineral metabolism are frequent in paediatric renal graft recipients. Furthermore, we found significant inverse associations between eGFR and phosphorus, Ca x P product 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 [5,24,25] we observed that 25% of the patients had phosphorus levels outside the target range. Hypophosphatemia was more prevalent than hypophosphatemia, 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 infants may already be in 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 FGF-23 secretion may also contribute to urinary phosphate losses after transplantation [5]. We were not able to test this hypothesis since PTH levels were missing in

86% of the observations and serum FGF-23 is not reported, but our data in older patients show a gradual decrease in hyperparathyroidism with time since transplantation. Since hypophosphatemia may impact on the mineralization of the growing bone, our findings point to a need for regular monitoring of serum phosphorus levels and consequent phosphate supplementation of hyperphosphataemic patients.

Overall 41% of our patients had elevated PTH levels, but they seem to improve with time since transplantation. When applying the same guidelines, the prevalence of hyperparathyroidism in our study is lower compared to North America [5] and Iran [24] (both 57%), and might reflect regional differences in PTH control, in keeping with data from paediatric patients on peritoneal dialysis [4].
Children who were on dialysis before receiving a transplant tended to have higher mineral levels than those who were transplanted pre-emptively, possibly due to the persistence of hyperparathyroidism [5,11,26]. We did, however, 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 [26]. Surprisingly, we found a decreased risk of hypophosphatemia among female patients, especially among the adolescents. Data from a recent study in adults suggest that women are more susceptible to hyperactivity of the parathyroid gland due to oestrogen action [26]. Immunosuppressive drugs can induce mineral effects. Corticosteroids can modulate calcium homeostasis leading to decreased intestinal absorption and increased urinary excretion [27]. Indeed, patients off steroids had a significantly lower risk of hypocalcaemia compared to patients using steroids.
We found inverse relationships of eGFR with serum phosphorus, Ca x P product and PTH levels, but we did not find any association for calcium. Previous studies reported mixed results. Bachetta *et al*. did not find any relationship between GFR and phosphorus, calcium, or Ca x P product [28]. However, only patients with GFRs above 30 ml/min/1.73m^2 were included in that study, whereas we found phosphorus and Ca x P product levels significantly increased only in children with eGFR levels below 29 ml/min/1.73m^2. Our findings are in keeping with data observed in native kidneys, where normal phosphorus levels are maintained until GFR drops below 30 [29] and with data from children with pre-dialysis CKD [30], where serum calcium was independent of GFR, whereas both phosphorus SDS and PTH levels were inversely associated only at lower GFRs.
In vitro, clinical and epidemiological studies have shown that increased phosphorus levels are independently associated with vascular calcification and mortality [31]. Findings both in animals and humans suggest that alterations in mineral metabolism can contribute to calcium phosphate deposition in the renal tubulointerstitium contributing to renal dysfunction [6,32]. Several studies have shown associations between serum phosphorus levels and decline in renal function in paediatric and adult CKD patients [7-9], and graft failure in adult

renal transplant patients [10,12-14]. We found an association between serum phosphorus, Ca x P product and an increased risk of graft failure. Serum phosphorus levels above recommended targets were independently associated with a higher risk of graft loss. Conversely, as 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. The association between elevated phosphorus levels and graft failure lost its significance after exclusion of eGFR levels below 20 ml/min/1.73m^2. This is not surprising, as the power is strongly reduced because many of the high phosphorus levels are also excluded. Moreover, 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, found an increased 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. Therefore, more research is needed to elucidate whether, and by which pathophysiological mechanisms, serum phosphorus levels may lead to an increased risk of graft failure. Wesseling-Perry *et al.* did not find any association of serum phosphorus and decrease in renal function or time to first rejection [5] within two years of follow-up. However, they observed a positive association for FGF-23. This difference might be due to the longer follow-up time in our study, as recent data in children with pre-dialysis CKD have shown that FGF-23 concentrations were increased early and progressively as GFR declined, and preceded any increase in serum phosphorus [30]. Unfortunately, FGF-23 is not reported in our registry, so we were not able to test whether levels were associated with graft failure.

Major strengths of our study are the size of the population and length of follow up, as well as the use of repeatedly measured mineral levels for the estimation of the risk of graft failure instead of using only a single baseline value. Limitations of our work include the lack of data on vitamin D or FGF-23, which might have provided greater insight into potential etiological mechanisms. Also, the reported medication use was very limited in our registry; therefore, we were not able to adjust the associations for medication use. Furthermore, the use of different analytical methods for determining mineral levels may have influenced our results. PTH levels in particular are subject to a high inter-method variability [33]. However, this variation is likely to be unrelated to whether or not mineral levels were outside the target range (non-differential misclassification) and may have led to an underestimation or dilution of the reported effects.

To summarise, abnormalities in mineral metabolism after paediatric 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. Our results emphasise the importance of controlling mineral levels after paediatric renal transplantation in order to timely correct mineral abnormalities that can impact on bone health and graft survival.

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